A STUDY OF THE TREATMENT OF METABOLIC ACIDOSIS IN DOGS

By

HOWARD JEFFREY MASS

Bachelor of Arts
University of Delaware
Newark, Delaware
1976

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1980

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 1984

Thesis
1984D
M414s
cop. 2

A STUDY OF THE TREATMENT OF METABOLIC

ACIDOSIS IN DOGS

Thesis Approved:

D. James E. Breazile
Thesis Advisor

George E. Burrows

James T. Blankenship

John A. Bantle

William H. Stewart

Dean of the Graduate College

1200613

ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. James E. Breazile, my major professor, for his suggestions and encouragement during the conduction of this research. I should also like to extend thanks to the members of my committee, Dr. George Burrows, Dr. James Blankemeyer, Dr. John Bantle and Dr. William Stewart for their assistance and comments in preparation of the final manuscript.

Special appreciation is extended to my family for their patience and support.

TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	3
	Body Water	3
	Disturbances of Volume and Osmalality	5
	Hemorrhage and Cardiovascular Changes	7
	TCA Cycle	9
	Glycolysis	10
	Gluconeogenesis	11
	Buffers	13
	Buffer Value and Efficiency	14
	Body Buffers	17
	Bicarbonate/Carbonic Acid System	18
	Hemoglobin and Plasma Proteins	19
	Renal Regulation of Acid Base Balance	25
	Potassium	26
	Metabolic Acidosis	27
	Anion Gap	27
	Fluid Therapy	29
	Objective	36
III.	MATERIALS - METHODS	37
	Preparation of Animals	37
	Experimental Procedure	38
	Sample Analysis	40
	Statistical Analysis	40
IV.	RESULTS	47
	Base Excess	47
	Bicarbonate Concentration	50
	PCO_2	50
	Anion Gap	55
	Packed Cell Volume	55
	Na+, Cl-, K+	55
	pH	55
	Dye Determinations	61

Chapter | Page

V. DISCUSSION. 63

Diagnosis of Metabolic Acidosis 63
Dilutional Acidosis 66
Fluid Shifts. 69

VI. SUMMARY AND CONCLUSIONS 70

SELECTED BIBLIOGRAPHY 72

APPENDIXES. 81

LIST OF TABLES

Table		Page
I.	Changes of Volume and Osmolality	6
II.	Blood Buffer Pairs	15
III.	Description of Treatments	39
IV.	Sample Analysis of Variance Treatments	45
V.	Changes in Base Excess	48
VI.	Changes in Bicarbonate Concentration	51
VII.	Changes in PCO_2	53
VIII.	Changes in Anion Gap	56
IX.	Changes in Packed Cell Volume	57
X.	Changes in Na^+	58
XI.	Changes in Cl^-	59
XII.	Changes in K^+	60
XIII.	Changes in Fluid Compartments	62

LIST OF FIGURES

Figure Page

1. Buffering of Carbon Dioxide by Hemoglobin. 20
2. Titration of Hemoglobin. 22
3. Mechanism of Buffering in Metabolic Acidosis 24
4. Experimental Design. 42
5. Changes in Base Excess 49
6. Changes in Bicarbonate Concentration 52
7. Changes in PCO_2. 54

CHAPTER I

INTRODUCTION

Metabolic acidosis, a condition resulting in a reduction of plasma bicarbonate, was first described in 1831 by W. B. O'Shaughnessy, who noted that the alkali content was increased in the stool and decreased in the blood of patients with cholera.[1] It was not until 1832, however, that the use of bicarbonate solutions was first described by T. Latta, who administered the solution intravenously to a dehydrated cholera patient.[2]

Bicarbonate gained popular usage after this success for treatment of acidosis, but fell into disfavor due to difficulties in sterilizing the solutions.[3] In 1932, Hartmann and Senn[4] reported the use of lactate as a bicarbonate precursor, while in 1949, Mudge, Manning and Gilman[5] recommended the use of acetate. However, unlike sodium bicarbonate, lactate and acetate require uptake by the cell and subsequent metabolism.[6-10]

If conditions exist in the animal, such as local tissue hypoxia, which might occur as a result of hemorrhage the metabolism of these anions may be inhibited.[11,12] In 1962, Schwartz and Waters supported the use of bicarbonate, rather than lactate, to treat acidosis resulting from tissue hypoxia and hemorrhage.[13]

Several studies have been conducted comparing the efficacy of sodium bicarbonate and sodium lactate to result in alkalinization.[14,7,3,9,10] The conclusion drawn from these studies was that, while sodium bicarbonate is the best choice, sodium lactate can result in varying degrees of alkalinization, depending on dose, over a period of time. However, these studies were conducted in normovolemic animals or under anesthesia.

It was the objective of this study to compare solutions containing either sodium lactate or sodium bicarbonate as the alkalinizing agent in a fluid replacement for a 20% blood loss in conscious dogs.

CHAPTER II

LITERATURE REVIEW

Water represents the most abundant component of the body and is the medium in which all life processes occur.[15] It is, therefore, necessary to maintain this fluid within relatively constant limits, and this was first recognized by Claude Bernard in 1850.

Changes in the water and electrolyte content of body fluids may lead to disruption of normal cellular function and eventual death. This section will deal with normal fluid volume and composition and changes that can occur in volume and osmolality of the extracellular fluid (ecf) space. Subsequent sections will detail the changes in cellular function that might occur as a result of fluid loss; the mechanisms of the body to maintain normal function and then principles governing fluid therapy.

Body Water

The total body water of an animal varies with the age, sex and fat content of the animal.[6,16,17,18] The percentage usually given for the total body water is 60% of the body weight (Kg), with a range between 55% and 70%.[15,19] Total body water is distributed into two major compartments, the extracellular fluid

(ecf) and intracellular fluid (icf) spaces. Total body water and ecf can be measured in the living animal by the dye-dilution technique, utilizing isotopes, polysaccharides or ions.[15,20,21,16,22,23]

Representing approximately 20% of the body weight, the ecf compartment can be divided further into three compartments: plasma, interstitial fluid and transcellular. The blood plasma comprises about 5% of the body weight (7% of the body weight is usually considered blood volume) and can be measured by Evan's blue or Cr^{51} or I^{131} labelled red cells or serum albumin.[24-27] Interstitial fluid volume, representing approximately 15% of the body weight, cannot be measured directly, therefore, it is measured by subtracting plasma volume from ecf volume. Intracellular fluid volume is also measured indirectly by subtracting ecf volume from total body water, and represents approximately 40% of the body weight.

The chemical composition of plasma and interstitial fluid are similar in that sodium is the principal cation, and chloride and bicarbonate the principal anions. Potassium and magnesium are the primary intracellular cations, whereas the phosphates and proteins are the principal anions. The major contributing factor to the difference between icf and ecf composition is the active transport of ions across the cell membrane. The major ion transported is sodium, which is actively moved out of the cells, while potassium is actively transported into cells. In most cases, anions are passively distributed.

It is the active transport of sodium out of the cell that

regulates the volume of the icf space. Also, as a result of being the dominant osmotic element of the extracellular fluid, sodium and its associated anions are the regulators of ecf volume. The maintenance of this sodium-potassium distribution has important implications in the administration of fluids that contain sodium and will be discussed in the section on fluid therapy.

Disturbances of Volume and Osmolality

Two terms, dehydration and overhydration, are common clinical terms used to describe clinical disturbances. However, to be more accurate, disturbances should include some description of the volume and osmolality change. The index typically used for osmolality change is the sodium concentration. Based on volume and osmolality, several changes can take place (see Table I). The first is isotonic expansion. Isotonic expansion is the proportional retention of sodium and water as occurs in edema or expansion of the ecf space with isotonic fluid. The fact that saline will remain essentially entirely in the ecf space remains an important consideration in fluid therapy. Hypertonic expansion occurs when sodium accumulates in excess of water, as in adminsitration of hypertonic saline. Hypertonic expansion is the accumulation of water in excess of sodium, as in water intoxication.

Contraction, or loss of fluid, from the ecf space can also be hypertonic, isotonic or hypotonic. Hypertonic contraction

TABLE I

TYPES OF CHANGES IN VOLUME AND OSMOLALITY

Extracellular Change	Clinical Example	Volume Change		Plasma	PCV	Plasma
		ECW	ICW	Sodium		Protein
Isotonic Contraction	Cholera	-	0	0	+	+
Hypertonic Contraction	Excess Sweating	-	-	+	0	+
Hypotonic Contraction	Renal Insufficiency	-	+	-	+	+
Isotonic Expansion	Saline Infusion	+	0	0	-	-
Hypertonic Expansion	Hypertonic Saline Infusion	+	-	+	-	-
Hypotonic Expansion	Water Intoxication	+	+	-	0	-

Direction of change is shown by symbols. 0 = no change; - = decrease; + = increase. ECW = extracellular water; ICW = intracellular water; PCV = packed cell volume. (from Goodman and Gilman's, The Pharmacological Basis of Therapeutics, pp. 848-884).

is dehydration in which there is a loss of water in excess of sodium. Examples of this type of dehydration include diabetes insipidus, excessive sweating and osmotic diuresis. Hypotonic contraction occurs when there is a loss of sodium in excess of water. Adrenocortical insufficiency is a common example of this type of dehydration.

Isotonic contraction results from sodium and water loss in isotonic proportions. Disorders commonly found include loss of fluid from the GI tract (vomiting, diarrhea) or loss of blood as in hemorrhage. Assuming no other metabolic disorders, fluid replacement with saline, for example, would be the appropriate therapy. However, as in the case of hemorrhage, reflex changes in the cardiovascular system can lead to alterations in tissue perfusion. These changes in perfusion can eventually lead to metabolic abnormalities that require more than simple volume expansion correction.

Hemorrhage and Cardiovascular Changes

There are a number of models that have been used to study the effects of blood loss on body fluid shifts, cardiorespiratory changes and the subsequent effects of fluid replacement on these parameters.[28] In the Wigger's model, blood is withdrawn until an arbitrary blood pressure is reached. This pressure is maintained for a given time by periodic reinfusion or withdrawal of blood from a heparinized reservoir. After a predetermined time at the reduced pressure, the blood is rapidly

reinfused in the dog.[11,28,29] It should be noted that this is a model of controlled hypotension and does not represent a natural model of hemorrhage. Any pressure adjustments by the animal are held in check by the investigator.[30,31] Other models that have been used are repetitive removal of given blood volumes over a predetermined period; a single, massive hemorrhage of a percentage of the blood volume or body weight; and total body exsanguination.[28,32] In these models, both splenectomized and/or nephrectomized dogs were used and the majority of the experiments were done under anesthesia.[11,32,33] Despite the diversity of models, in 1965, Swan reported the status of a standardized experimental model designed to study the dynamic response of the animal to a single, massive hemorrhage.[34] The changes in the cardiovascular system following the loss of blood involve mechanisms designed to maintain perfusion to the heart, lungs and brain at the expense of flow to areas such as the gastrointestinal tract. The sympathetic nervous system, through the release of norepinephrine and epinephrine, result in vasoconstriction and a change in blood pressure. This sympathetic nervous system mediated vasoconstriction is of great importance to the animal in the maintenance of blood pressure after hemorrhage.[16,11,35] In 1921, Meek and Eyster[36] showed that morphinized dogs were able to compensate for blood loss averaging 2% of the body weight without significant changes in heart rate, arterial pressure and cardiac filling. It has also been demonstrated that a rapid loss of approximately 50% of the blood volume is required to cause death in normal

animals. If the nerves of the reflex are sectioned, a rapid loss of only 10% of the blood volume proves fatal.

Besides the reflex vasoconstriction, the shift of fluid from the interstitial space to the vascular system is another mechanism to maintain adequate tissue perfusion.[37,38,39] This process can account for restoration of approximately 80% of the fluid loss within hours.[28,32] This fluid shift and any new fluid entering the system (e.g., intravenous fluid administration) will dilute the plasma proteins. This protein will eventually be replaced and a normal concentration restored as a result of drainage from the lymphatic system.[37]

As previously mentioned, a consequence of the decreased tissue perfusion is a change in cellular metabolism.[40] This change in cellular metabolism occurs in the three major energy processes of the cell: Glycolysis, the tricarboxylic acid (TCA) cycle, and gluconeogenesis.[41-43] The pathways and its regulators will first be considered.

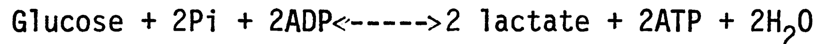
TCA Cycle

The TCA cycle, first postulated by H. A. Kregs in 1937, is a cyclic sequence of reactions that functions as the main pathway for oxidation of carbohydrates.[44] The first step where citrate is converted to isocitrate can proceed anaerobically. It is the next step, conversion to alpha ketoglutarate, which is the first aerobic step.[44,45] Therefore, following a decrease in tissue perfusion, the development of a local tissue hypoxia will

inhibit the TCA cycle.

Glycolysis

Glycolysis is a catabolic pathway, breaking down glycogen or glucose to either lactic or pyruvic acids.[44] It is an anaerobic process and the most common fuel is glucose, although some organisms can use fatty acids. The overall balance equation for the process is:



$$\text{Glucose} + 2\text{Pi} + 2\text{ADP} \longleftrightarrow 2\ \text{lactate} + 2\text{ATP} + 2H_2O$$

where Pi is inorganic phosphate. There are two major stages of glycolysis, the first stage preparing glucose for catabolism by phosphorylation. It ends by cleaving the molecule into two, three carbon sugars. The second stage converts the three carbon sugars into lactate, via pyruvate. When pyruvate is formed, the redox state of the cell determines the subsequent reaction direction. The reoxidation of NADH by the transfer of H^+ and its electron to oxygen through the respiratory chain is inhibited in an anaerobic state.[12] Under these conditions, pyruvate is reduced to lactate by lactate dehydrogenase (LDH). Tissues under anaerobic conditions, therefore, produce increased quantities of lactic acid,[12,44] and hence, a lactic acidosis develops. This increase in lactic acid due to hypoxia, has been show to occur by Huckabee[95] even before any significant cardiovascular changes, such as a change in heart rate or blood pressure.

Another factor responsible for the increased lactate is the

inhibition of the enzyme pyruvate dehydrogenase.[8] This enzyme is responsible for the conversion of pyruvate to acetyl CoA, a step necessary for glucose oxidation via the TCA cycle.[44]

A major regulatory point in glycolysis is the phosphofructokinase (PFK) enzyme catalyzed reaction. This step is regulated by the concentration of ATP and ADP in the cell.[44] Increased [ATP] will inhibit this enzyme; high [ADP] will stimulate enzyme activity. However, the decrease in intracellular pH, which occurs during anaerobiosis, will inhibit PFK activity despite the higher concentrations of ADP seen during hypoxia.[8,45,43]

Gluconeogenesis

An important response to the energy and substrate deficits of inadequate tissue perfusion is the stimulation of gluconeogenesis.[44] Since glucose is the only substrate that supplies energy to the cell under anaerobic conditions, alternative fuel sources must be converted to glucose. The metabolism of lactate, the major product of anaerobic metabolism, will now be discussed in its relation to gluconeogenesis.

Lactate is converted to pyruvate in a reaction catalyzed by LDH, which is a reversal of the reduction of pyruvate to lactate.[45] There are two isozymes of LDH, each with its own affinity for pyruvate. Those tissues with a high enzyme affinity for pyruvate do not easily convert lactate into pyruvate. In these tissues (e.g., skeletal muscle), the LDH reaction has a greater tendency to proceed from pyruvate to lactate.[44,45] However, in

in conditions of high ecf lactate concentration, skeletal muscle can utilize lactate as an oxidative substrate. In other tissues (heart, liver), the reaction will proceed favorably in the direction of pyruvate.[44]

The next few steps involve the conversion of pyruvate to phosphoenolpyruvate (PEP) via oxaloacetate. The enzymes involved are pyruvate carboxylase and PEP carboxykinase. The same enzymes of glycolysis are followed in reverse order, until the formation of fructose 1,6-diphosphate. At this point, the enzyme fructose 1,6-diphosphatase removes a phosphate group to form fructose 6-phosphate.

Gluconeogenesis is inhibited by a decrease in intracellular pH, at the pyruvate carboxylase and PEP carboxykinase steps. A low energy state (increased [ADP]) inhibits the enzyme fructose diphosphatase.[45-49]

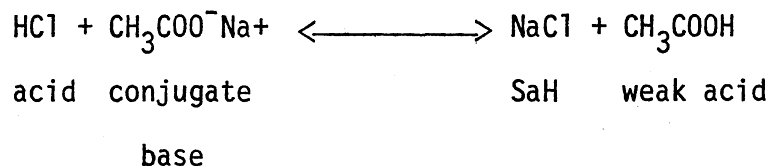
In summary, the reduced tissue perfusion resulting from hemorrhage, leads to specific metabolic changes. Under anaerobic conditions, glycolysis becomes the primary source of energy, resulting in the production of large amounts of lactic acid. Inhibition of gluconeogenesis and the TCA cycle by the decrease in intracellular pH, results in underutilization of lactate, leading to a clinical acidosis.[50-52]

The clinical manifestations of blood loss can be followed in changes in blood gases, plasma potassium levels and the anion gap (the difference between the concentrations of the cations, sodium and potassium, and the anions, chloride and bicarbonate).[53]

The titration of organic acids in extracellular fluid leads to a decrease in bicarbonate concentrations and pH. To maintain the pH within limits compatible with life, there are three mechanisms, each varying with the rapidity of the response. The first is a chemical buffering that reacts immediately with added acid or base.[15,16,21] The second mechanism is the respiratory system which regulates the carbon dioxide levels (PCO_2) of body fluids.[18] The final mechanism, the renal mechanism, is a relatively slow process which regulates both hydrogen ion and bicarbonate concentrations of extracellular fluids.[21]

Buffers

A buffer system is a solution containing a weak acid along with its conjugate base, which functions to resist changes in pH when a stronger acid or a base is added.[54-56] A typical reaction of buffer action can be understood in terms of the following equation:



This equation states that the conjugate base of acetic acid (sodium acetate) binds the hydrogen ions dissociated from the strong acid, to form a weak acid and a neutral salt.[57] There is, however, a definite limit to how much acid or base can be added to a buffer solution before an appreciable change in pH will result. This limit is known as the buffer capacity and is set by

the original quantities of the weak acid and its conjugate base, since one or the other is consumed in any buffer reaction.[4]

Buffer Value and Efficiency

In 1914, Max Koppel and K. Spiro introduced the concept of the buffer value of weak acids and bases,[78] although it was much later that Van Slyke added a physiologic mechanism as an important perspective to the buffer studies.[78]

The efficiency of a buffer is determined by the relationship of the pKa and pH of the solution.[16] A buffer is most efficient at its pKa ± 1 pH unit.[55] The addition of acid or base when the buffer is within this range results in a smaller change than at other pH values. The efficiency is greater at this point because: 1) the concentration of the acid and base are equal; and 2) the equilibrium does not favor a reaction in either direction.[52] Table II lists various blood buffer pairs, the pKa and the base/acid ratios at several physiologic pH values. It can be seen that even at a pH of 7.0 the lactate/lactic acid ratio is 1258/1. For sodium lactate to act as a base, the reaction

where A^- is the conjugate base of HA, must take place. However, at pH 7.0, only 1/1258th of the sodium lactate present would be capable of this reaction. An important concept relating to buffering efficiency is the relationship between the pKa and the affinity of the conjugate base for protons. The general rule is, the lower the pKa, the stronger the acid and the weaker the base.

TABLE II

BLOOD BUFFERS AND ACID/BASE RATIOS AT SEVERAL PHYSIOLOGIC pH VALUES

Buffer Pair	pKa	Ratios at pH Values: 7.0	7.4	7.6
ammonia/ammonium	9.37	.004	.01	.02
$HPO_4^{=}/H_2PO_4^{-}$	6.8	1.5	4	6.3
HCO_3^{-}/H_2CO_3	6.1	7.9	20	32
β-hydroxybutyrate/ β-hydroxybutyric	4.8	158	398	630
propionate/propionic	4.8	158	398	630
acetate/acetic	4.5	316	798	1258
lactate/lactic	3.9	1258	3100	5000
acetoacetate/ acetoacetic	3.8	1584	3980	6300

The values presented are the base/acid ratios at the listed pH values. The denominator in all cases is 1 (eg.,.004/1).

When comparing acetic acid and the lactic acid with pKa's of 4.5 and 3.9 respectively, this rule would predict that lactic acid, being a stronger acid than acetic, the conjugate base sodium acetate has a greater proton affinity than sodium lactate. If a proton transfer reaction was to occur between sodium lactate and acetic acid, the following reaction would take place:

acetic acid + sodium lactate   acetate + lactate acid

A general form of the two preceding reactions might be stated as follows using the symbols: AH, representing the stronger acid, and BH, representing the weaker acid; A^-, B^- as the conjugate gases of the corresponding acids. In the reaction

$$AH + B^- \longleftrightarrow A^- + BH,$$

if the pKa of AH is greater than the pKa of BH, the general concept is that an acid cannot be effectively buffered by the salt of a stronger acid (or one with a lower pK value).

A second important consideration is the ability of animal to alter the base/acid ratio by physiologic mechanisms.[15,18] This is an important mechanism in the bicarbonate - carbonic acid system. Referring to Table II, it would appear by virtue of the pKa, the phosphate system would be most efficient of the physiologic buffers, for buffering at pH 7.4. However, since the bicarbonate - carbonic acid system is easily and rapidly regulated by respiratory alterations of the levels of carbon dioxide in the blood (pCO_2, measured in mmHg), this buffer system is very efficient for buffering at pH 7.4 in extracellular fluid.[19]

Another way of expressing the efficiency of a buffer is by its buffer value. The buffer value of a solution at any pH is defined as the negative slope of titration curve at that pH.[19] The slope is the amount of acid or base that is needed to produce a pH change of 1 unit.[55] The steeper the curve, the greater the amount of acid needed to change the pH. The buffer value is usually expressed as mmols per liter per pH unit. For any weak acid or base, this value is 0.575 per pH unit for each ionizing group on the molecule, regardless of its structure or Ka.[58] For substances with multiple ionizing groups, such as the proteins, hemoglobin and the polyprotic acids, the pH range of operation is an important consideration.[19] This will be discussed below in a consideration of the hemoglobin - plasma protein buffers.

Body Buffers

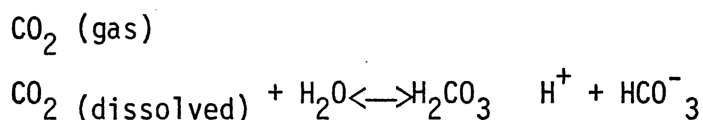
There are a number of buffer systems within both the extracellular and intracellular fluids.[15,21] The intracellular buffers are the various phosphates, organic anions such as proteins, the bicarbonate-carbonic acid system and hemoglobin.[18] Since intracellular fluid is approximately 67% of total body water, these buffers represent a considerable quantity of buffer chemicals and they play an important role in the maintenance of the pH of body fluids.

Extracellular buffers include plasma proteins, and the weak acids and bases of the blood, such as $NaHCO_3/H_2CO_3$, $Na_2HPO_4/$

Na_2HPO_4, sodium acetate/acetic acid and sodium lactate/lactic acid.[21]

Bicarbonate/Carbonic Acid System

The discovery of the critical role of the bicarbonate/carbonic acid buffer system paved the way for the elucidation of acid base physiology and an appreciation of the clinical disturbances of acid base metabolism.[52] The most important discovery in the acid base field was made by L. J. Henderson in 1908.[52] From observations made in the previous century and his own observations on the bicarbonate and phosphate buffers, he recognized that the bicarbonate/carbonic acid buffer plays a central role in stabilizing the acid base status of the animal.[79] He pointed out that the response of this system to the introduction of acid involves a rapid reduction in carbonic acid, achieved by removal of carbon dioxide by the lungs.



$$CO_2 \text{ (gas)}$$
$$CO_2 \text{ (dissolved)} + H_2O \longleftrightarrow H_2CO_3 \quad H^+ + HCO^-_3$$

This is an important feature of this buffer system and is a reason why this system is efficient despite a pKa of 6.1.[15,19]

The pH of this system can be determined by the Henderson-Hasselbalch equation:

$$pH = pKa + \log \frac{\{H_2CO_3\}}{\{H_2CO_3\}} \quad \text{or since } \{H_2CO_3\} = .031 \times PCO_2$$

$$= pKa + \log \frac{\{H_2CO_3\}}{.031 \times PCO_2}$$

If a closed system is present, where the concentration of acid increases as H^+ reacts with the base, addition of 10 mEq of acid will lower the pH from 7.4 to 6.2.[1] However, in an open system, where excess carbon dioxide is allowed to escape while PCO_2 remains constant, the pH changes from 7.4 to 7.2. The constant PCO_2, in vivo, is maintained by production of CO_2 by the cells, and the regulation of respiratory ventilation by pH of extracellular fluids.[19] The buffer value, at a constant PCO_2 can thus be calculated and is found to be 2.3 per pH unit, a value four times that of ordinary buffers.[19]

In this open system, unlike other buffer systems, diluting the system will change the buffering ability and pH. Adding water is equivalent to adding acid (dilutional acidosis) and removing water is equivalent to adding base (concentration alkalosis).[15] Thus, changes in body water volume can have acid base effects.

Hemoglobin and Plasma Proteins

Figure 1 is a diagram of the role of hemoglobin and erythrocyte in buffering the carbon dioxide released from tissues. In this example, carbon dioxide rapidly diffuses into the erythrocyte. A fraction remains dissolved while the majority combines with water and through a reaction catalyzed by carbonic anhydrase, carbonic acid is formed. Carbonic acid dissociates into the bicarbonate and hydrogen ions. The bicarbonate ion leaves the cell in exchange for chloride, while the hydrogen ion is buffered by hemoglobin.[15,59] Titration of 1 mmol per liter

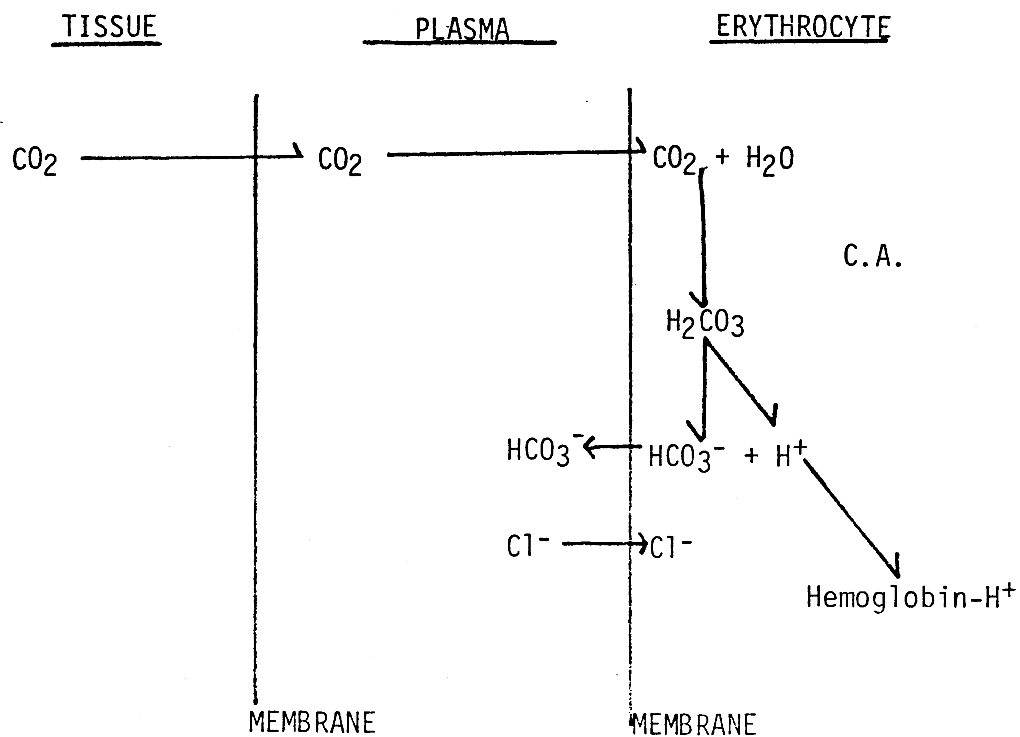


Figure 1. The Buffering of Carbon Dioxide by Hemoglobin
C.A. = Carbonic Anhydrase

solution of oxhemoglobin and hemoglobin, over the pH range 7.0 to 7.8 (Figure 2) gives a buffer value (or slope) of 3.0 per unit change pH.[19,60] The buffer value of hemoglobin in a liter of blood is 22.7.[19]

The buffer value of whole blood can be found by the addition of the buffer values for hemoglobin and plasma proteins. As with hemoglobin, the buffer value of the plasma proteins is dependent upon the concentration of plasma protein. According to Van Slyke et al,[58] the buffer value of protein, which is measured in mmol/gram/unit pH is 0.1. Assuming a normal protein concentration of 7 grams per 100 ml and a hematocrit of 45, one liter of plasma has a buffer value of 3.9. Adding this to 22.7 (buffer value of hemoglobin) gives 26.6 as the buffer value of whole blood.[19]

These experiments are a reflection of the buffering capability of extracellular fluid as a whole. Since interstitial fluid is essentially protein free, the buffering of carbonic acid in extracellular fluid is primarily the responsibility of plasma proteins and hemoglobin. Titrations of whole blood with a noncarbonic acid will involve all the buffer systems, including the bicarbonate/carbonic acid system.[19]

The change in buffer value of true plasma removed from erythrocytes prior to changes in pCO_2 or before any significant reaction with hemoglobin versus separated plasma allowed to equilibrate with carbon dioxide and hemoglobin (of the erythrocytes), has an important implication in acid base disorders. As mentioned

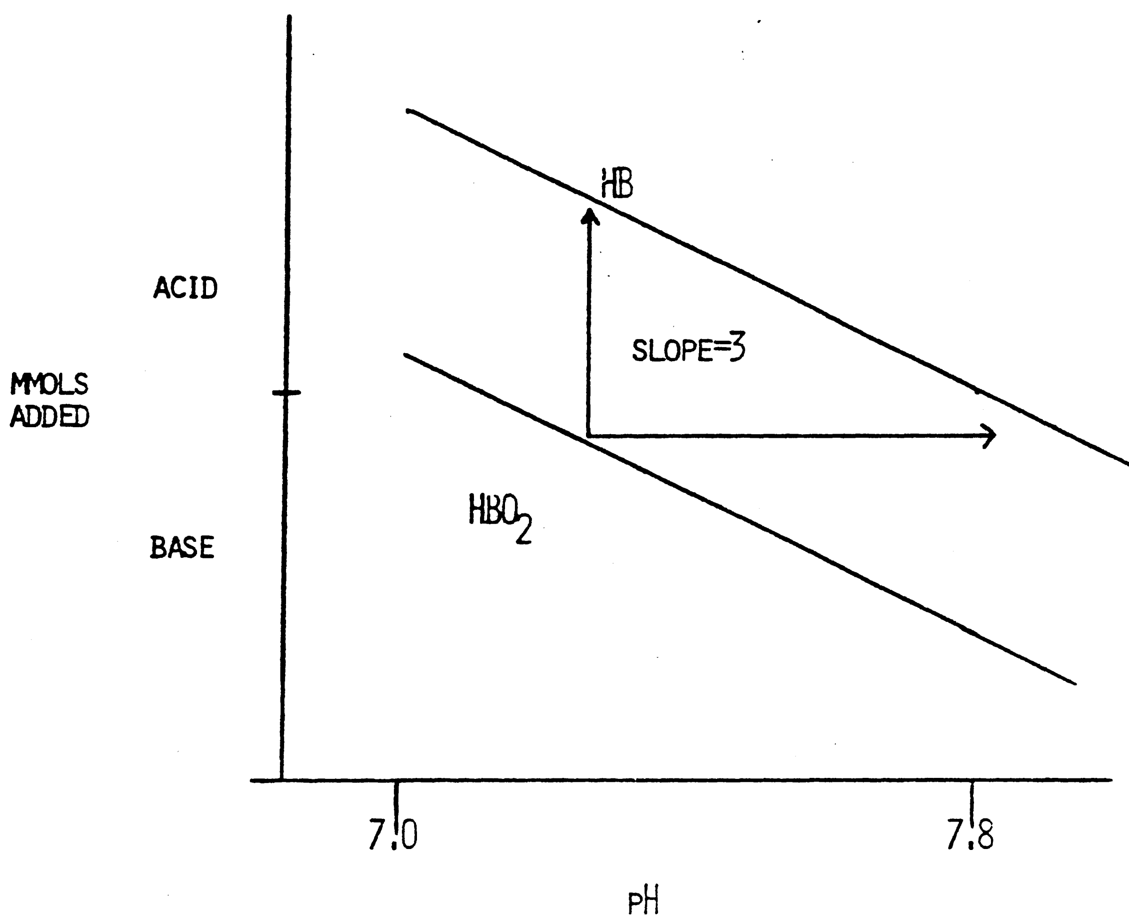


Figure 2. Titration of Hemoglobin (HB) and Oxyhemoglobin (HBO_2)

(From: Woodbury, D.M. Physiology of Body Fluids. In: Ruch and Patton, Physiology and Biophysics II Circulation, Respiration and Fluid Balance, 1974, pp. 450-479).

previously, addition of acid to the system will result in a movement of bicarbonate ions out of the erythrocyte, which will increase the availability of base outside of the cell. This movement will also tend to dampen any change in extracellular sodium bicarbonate due to consumption by the acid. The implications in acid base disorders is that, by virtue of this dampening process, a mild increase of acid may not result in a change in the extracellular bicarbonate concentration, but does result in a reduction of intracellular HCO_3^-. More important is the idea that when changes in extracellular fluid bicarbonate concentrations are noted, as by a decrease in the base excess, the intracellular reserves are already partially depleted. This will be discussed further in the fluid therapy section. The role of other intracellular buffers will be discussed below.

In discussing the buffering of metabolic acidosis, the origin of the acid needs to be considered. In the classic study by Swan and Pitts,[61] the infused acid was hydrochloric acid. It has been shown, however, that the response to organic acid infusion is slightly different from that to a mineral acid.[14]

If a strong mineral acid such as hydrochloric acid is infused, approximately one percent is buffered by the extracellular fluid proteins. The hydrogen ions are accepted by the proteins, displacing cations, primarily sodium, resulting in an increased sodium chloride concentration. Approximately six percent of the buffering results from an exchange of extracellular chloride for intracellular bicarbonate involving the erythrocytes. Forty-three

Intracellular Fluid		Extracellular Fluid	
Na^+	$\rightleftharpoons$	H^+	36%
K^+	$\rightleftharpoons$	H^+	15%
HCO_3	$\rightleftharpoons$	Cl^-	6%
		total intracellular buffering	57%

$$HCO_3^- + H^+ \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O \quad 42\%$$

Protein + H^+ 1%

total extracellular buffering 43%

Figure 3. Mechanism of Buffering in Metabolic Acidosis. This figure represents the mechanism of buffering after infusion of a strong mineral acid (HCl). The intracellular buffers account for the majority of buffering (57%); whereas the extracellular beffers account for approximately 43% of the buffering.

(From: Swan, R.C., Pitts, R.F. Neutralization of infused acid by nephrectomized dogs. J. Clin. Invest. 34:205-212, 1955)

percent of extracellular buffering occurs with the sodium bicarbonate system, resulting in an increased PCO_2. The greatest fraction of acid is buffered by the exchange of cellular potassium (15%) and sodium (37%) for extracellular hydrogen ions.[21,61]

The changes occurring after infusion of an organic acid, such as lactic acid, are somewhat different than those described above for HCl infusion.[14] During infusion of lactic acid, although the concentration does initially rise, it rapidly falls after the infusion is discontinued. This rapid disappearance is attributed to its free diffusibility within total body water and to cellular metabolism. It has been shown that within ten minutes of injection, 40% of the lactic acid dose is metabolized. The buffering by extracellular bicarbonate only accounts for approximately 33% of lactic acid disappearance. These experiments were performed in normal animals with no evidence of tissue hypoxia. Under anaerobic conditions, however, the metabolism of lactate is blocked by enzyme inhibition due to pH changes.

Renal Regulation of Acid Base Balance

The discussion so far has focused on the chemical compensation of acidosis or alkalosis. Respiration is the second line of compensation because changing the respiratory rate will alter the PCO_2.[15] This, in turn, will alter the bicarbonate-carbonic acid ratio and thus the pH. (See previous discussion of the bicarbonate buffer system for more detail.)[21,19]

The third mechanism of acid base regulation is the renal mechanism, which occurs over a long period of time. The renal response can be divided into separate phases, as follows: 1) the reabsorption of sodium bicarbonate; (2) the excretion of titratable acids; and 3) the excretion of anions in combination with NH_4^+ instead of Na^+.[20]

Potassium

Associated with changes in blood acidity are changes in the plasma $[K^+]$. It is widely accepted that acidosis is associated with K^+ release from the cells with an increase in plasma [K].[62] There have also been attempts to quantitate the changes in plasma K as a function of pH. According to Scribner et al[65] and others[63,64] a change in pH of .1 unit leads to a change of 0.6 mEq/l of plasma potassium in the opposite direction. This value was derived through the study of mineral acid induced acidosis.

Recent studies using organic acids indicate that this generalization may not be applicable to clinical acidosis. In fact, it appears that differences exist among the acid base disorders with respect to changes in plasma potassium concentration.[66-70] These changes appear to depend on factors other than pH, such as plasma osmolality, adrenergic activity, insulin levels, liver activity and plasma bicarbonate concentration.[63,71,72,73] The discussion will focus on the changes occurring during metabolic acidosis.

Metabolic Acidosis

The acidosis as a result of infusion of a mineral acid, such as hydrochloric or ammonium chloride, consistently leads to an increase in plasma potassium. In sharp contrast, acidosis as a result of organic acid (lactic acid, acetic acid, b-OH Butyric and methylmalonic acids) infusion was not associated with a significant change in plasma potassium concentration.[63,73,74]

Anion Gap

In addition to serum electrolyte and blood gas determinations, calculation of the anion gap is becoming a useful clinical tool.[75,76] The anion gap is the difference in the sum of the routinely measured cations (sodium, potassium) and the sum of the routinely measured anions (chloride, bicarbonate):[52,53]

$$\text{anion gap} = ([Na] = [K]) - ([Cl] + [HCO_3])$$

On the basis of the normal values of the ions, in mEq/l, the difference between the concentrations of cations and anions ranges from 10 to 16 mEq/l.

$$\begin{aligned}\text{anion gap} &= ([Na] = =[K]) - ([Cl] = [HCO_3]) \\ &= (145 + 5) - (110 + 25) \\ &= 150 - 135 = 5 \text{ mEq/liter}\end{aligned}$$

The difference of 15 mEq/l reflects a concentration of cations in addition to Cl and HCO_3^- which are unmeasured in routine examination of plasma. The concentration of unmeasured anions (phosphate, sulfate, organic acids, proteins) generally exceeds

that of the unmeasured cations (magnesium, calcium).

An increased or decreased anion gap may result from a change in the unmeasured anions or a change in the measured cations.

The primary usefulness of the anion gap lies in its delineation of the various causes of metabolic acidosis.[75] An increased anion gap occurs in metabolic acidosis produced by diabetic ketoacidosis and lactic acidosis. In the former condition, production of acetoacetic and B-hydroxybutyric acids exceed their rates of metabolism and excretion. Lactic acidosis tissue hypoxia which decreases aerobic oxidation of glucose in the tricarboxylic acid cycle will lead to increased lactic acid levels of plasma. This increased liberation of lactic acid, which exceeds its rate of conversion by the liver to glucose, leads to the increased anion gap. Acute renal failure may also be associated with an increased anion gap.[75,76,77]

Conditions associated with a normal or decreased anion gap in metabolic acidosis are renal and gastro-intestinal loss of bicarbonate, hyperchloremic acidosis and dilutional acidosis. In the decrease of the anion gap during acidosis, it has been demonstrated by Androgue et al[75] that approximately 40% of the observed decrease in the gap is due to plasma proteins. As hydrogen ions are added to plasma, the negative charge on the proteins will decrease, resulting in a decrease of the anion gap.[75]

In summary, the changes that occur as a result of a metabolic acidosis are: a decrease in plasma bicarbonate, an increase in the negative base excess; an increase in the anion gap

(>20 mEq/l) and plasma potassium; and a decrease in PCO_2 due to respiratory compensation.

To this point, the discussion has focused upon the changes that occur as result of hemorrhage and the mechanisms of the body designed to minimize the changes in acid base status. The discussion will now turn to fluid therapy and the basis of treatment of the metabolic disorders that result from hemorrhage.

Fluid Therapy

The aim of fluid therapy, in cases where there is a decreased circulating blood volume, is the re-establishment of adequate tissue perfusion to prevent cell death.[80] Associated with this is the correction of the acid base status of the animal.

There are a number of commercially available solutions for body fluid volume restoration ranging from those containing only sodium chloride (normal or physiologic saline) to polyionic solutions (Ringer's and lactated Ringer's solutions) to dextrose in water.[17] In choosing a solution for administration, the nature of the deficit in either volume and/or electrolytes is usually considered.

As previously mentioned, loss of blood will result in reflex cardiovascular changes designed to maintain perfusion of the heart, brain and lungs at the expense of the cutaneous and splanchnic circulations. This decrease in perfusion in these areas will result in local tissue hypoxia and lead to the development of metabolic acidosis.

The buffer systems of the body will, in part, compensate for the acidosis,[15,19] and shifts will tend to compensate for the blood loss.[39] To assure adequate perfusion, however, fluid replacement may be a necessary step.[17] The best replacement for the blood loss would be whole blood.[81] If this is not available, then plasma should be considered, followed by plasma expanders, such as dextran. If none of these are available, balanced electrolyte solutions are the next choice.[82] There are a number of these available, but the discussion will focus on the following commonly used solutions: normal or physiologic saline, lactated Ringer's and 5% dextrose in water (D5W).

Physiologic or normal saline is a 0.9% solution of sodium chloride (150 mEq/l each ion). In terms of expanding the ecf space, it is efficient. Its first reported successful use in the treatment of fluid loss was in 1891.[83] However, a problem associated with the use of saline is the dilution of the bicarbonate buffer system, leading to a dilutional acidosis.[84-87] The acidifying effect of saline has been debated for many years,[84,85] first being proposed by Peters and Van Slyke in 1931[88] and demonstrated by Shires and Holman in 1947.[87] Although it is now established that saline infusion can result in an acidosis,[84,89] there is still disagreement as to the amount necessary to result in a significant lowering of the plasma bicarbonate.[86,87] Garella et al,[86] state that the ecf space needs expansion by approximately 40% over its original volume in normal animals to result in a significant acidosis. It has been suggested by

Garella et al[87] and others[90] that the minor changes in pH and bicarbonate that occur following saline infusion are a result of intracellular buffering. It should be noted, however, that these investigations were carried out in normovolemic animals with no apparent acid base disturbances. The use of normal saline would appear to be contraindicated for use in animals with a metabolic acidosis, whose buffering systems are already being challenged by the accumulation of organic acids.[41] The use of a buffer base with the saline, such as bicarbonate, would be a logical alternative. This will be discussed in further detail below.

In the case of D5W, a similar condition of dilutional acidosis occurs. However, the degree of dilution is less severe due to entry of the glucose and water into the cell.[17] The glucose solution partitions itself according to the icf/ecf water volumes, with 2/3 of the added solution entering the cells: 1/3 remaining in the ecf space. Because glucose distributes to the total body water pool it has less value as a blood loss replacement than does saline. Glucose solutions dilute both icf and ecf. Dilutional acidosis of both compartments result, whereas, in saline infusion, only ecf dilution results.

Lactated Ringer's solution is an isotonic, polyionic solution introduced in 1932 by Hartmann and Senn.[4] It is now widely used as a therapy for several reasons. The first is that the sodium lactate is assumed to act as a buffer in cases of acidosis. This point will be discussed below. Based on this buffer base concept, Ringer's solution would only theoretically result in a

temporary dilution of plasma bicarbonate, thus avoiding dilutional acidosis. The final reason is that it provides electrolytes, namely K^+ which may be lost during acidosis. However, during acidosis, there is a rise in plasma potassium, and increasing plasma K in the presence of an unknown renal function may lead to complications, such as cardiac arrhythmias.

As mentioned previously, even in cases where there are no metabolic disorders, the use of a buffer base such as sodium bicarbonate in fluid replacement therapy is important to prevent a dilutional acidosis. In these cases, the base requirement to prevent dilution of the plasma bicarbonate would be the normal concentration of bicarbonate, or 25 mEq/l.[17] Because the apparent HCO_3^- space is approximately equal to 83% of total body water, the use of 25 mEq/l of fluid is required for either saline or glucose to prevent dilutional acidosis.

If, however, there are signs of acidosis, the base requirements will change. One consideration is the type of alkali to use in the correction of the acidosis.[9,91] There are several possible alkalinizing agents, namely the sodium salts of the following weak acids: lactic, carbonic, acetic, succinic and gluconic.[92,93,91,10,9] Of these compounds, sodium bicarbonate is indicated for acute severe metabolic acidosis, because of its rapid effect when given intravenously.[9]

This is based first of all, on the physical chemical properties of the bicarbonate-carbonic acid buffer system. Having a pKa of 6.1, this system would be most effective within the normal

pH range of most organisms, and would therefore accept hydrogen ions from essentially all metabolic acids. (Note: Recall it is the ease with which the concentrations of carbonic acid can be changed via respiration that allows bicarbonate to be effective against phosphate, pKa 6.8.) On the other hand, the pKa of the lactate/lactic acid system is 3.9. Therefore, at a pH of 7.4, almost 100% of the lactic acid would exist as sodium lactate. This prevents it from accepting hydrogen ions and thus act as a buffer.

A second reason why sodium bicarbonate is considered a more effective alkalinizing agent is that it does not require metabolism to work.[9,10,91] According to these investigations and other[7,41,82,93,94] agents such as sodium lactate are effective as buffers only after metabolism to sodium bicarbonate. It is the generated sodium bicarbonate which is the effective buffer agent. Both Hartsfield[9,10] and Kirkendol[93] have shown an increase in sodium bicarbonate following infusions of sodium acetate or sodium lactate, which is consistent with this idea. In the study by Kirkendol et al[93] sodium acetate increased plasma bicarbonate after 20 minutes of infusion, while sodium lactate increased plasma bicarbonate after 40 minutes of infusion.

However, if lactate or acetate is metabolized the products formed are carbon dioxide and water and not sodium bicarbonate.[14,44] This was demonstrated by Mithoefer and Karetzky[14] who infused lactic acid in anesthetized dogs and found an increase only in the PCO_2. The PCO_2 values were also found to increase in the

Hartsfield[9,10] study, which would indicate that lactate or acetate were metabolized to CO_2. (NOTE: All experiments were carried out in normovolemic animals.)

A possible source of the rise in bicarbonate seen in the Hartsfield study or Kirkendol investigation[9,10,93] could be ion exchange. As the lactate (or acetate) ion would enter the cell, an intracellular anion would need to leave the cell to maintain electroneutrality. The anions that could take part in this exchange would be either chloride or bicarbonate.

A final reason for choosing sodium bicarbonate as an alkaline buffer fluid therapy in metabolic acidosis is that the acidosis resulting from blood loss and/or tissue hypoxia is a lactic acidosis.[41,95] This type of acidosis, resulting from volume depletion, was described by Clausen in 1925[97] and by Hartmann and Senn[4] in 1932. Huckabee[95] and Phillipson et al[96] also described lactic acidosis resulting from a decreased tissue perfusion. The mechanism of the increase in plasma lactate concentrations reresults from overproduction and underutilization of lactate. The administration of a solution that contains lactate as a buffer system is ineffective in buffering its own acid or base.

The proponents of lactate therapy state, however, that, although there is a metabolic consideration, increasing tissue perfusion, which will, in itself, correct any metabolic abnormalities, also adds an additional dilutional acidosis to complicate therapy. Canizaro et al[99] have stated that adding lactate solution to an already overloaded system does not have a significant

effect and is probably not detrimental to the animal. In a series of 23 unconscious dogs, bled 60% of their calculated blood volume, mean blood lactate increased from a control level of 13 mg% to 91 mg% within 30 minutes following bleeding. When treated with lactated Ringer's the blood lactate concentrations increased slightly to 100 MG%.[99] Blood gas or pH measurements were not available but based on blood lactate levels, the treatment would seem to be hindering the objective of decreasing blood lactate levels.

Once the clinician has decided on the buffer base to use, the next question to answer is the quantity of base to add.[100] (Sodium bicarbonate is the example which will be used in this discussion.)

The severity of the metabolic acidosis is usually estimated by determination of the base excess.[84] The base excess is usually calculated from the plasma concentration of bicarbonate and pH.[52] It should be realized that the base excess only represents the sodium bicarbonate excess, with a positive excess associated with alkalosis; a negative excess with acidosis.[57,44] The amount of bicarbonate to be administered, in mEq/l, is usually calculated from the formula of Mellemgard and Astrup.[101] This formula estimates the bicarbonate replacement in a nonrespiratory acidosis to be 0.3 x body weight (kg) x base excess. The term 0.3 x body weight (kg) somewhat overestimates the ecf space.[17,100] This formula may prove inadequate for two reasons.[100] The first is that it does not include the 25 mEq/l maintenance dose or normal concentration of bicarbonate needed to prevent dilutional acidosis. The other reason is that the base excess does not

account for buffering by the other buffering systems of the body (e.g.,intracellular buffers account for 57% of the buffering in a mineral acidosis) and the dampening of bicarbonate loss by hemoglobin.[61] Depending on the severity and longevity of the acidosis, the bicarbonate concentration may fail to rise when corrected in this manner.[100] It has been demonstrated by Garella et al[100] that bicarbonate will distribute itself in a apparent fluid space equal to 50% of the body weight, and in some cases the requirements may theoretically exceed 100% of the body weight due to the need to restore other buffer systems.

Objective

Based on the pKa values for sodium lactate and bicarbonate, it is hypothesized that sodium bicarbonate would act as a more efficient proton acceptor for use in fluid replacement to correct a metabolic acidosis produced by a 20% blood loss.

The acid base status will be monitored by changes in blood gases, specifically a decrease in plasma bicarbonate, or an increase in the negative base excess. Other changes that could occur in the blood gas status of the animal would be a decrease in pH and/or PCO_2; the decrease in the latter index is a result of respiratory compensation. Subsequent analysis of the anion gap will be used to confirm the presence of an acidosis (>20 mEq/l). To remove any influence of anesthesia on compensation mechanisms or on the acid base status of the animal, these experiments will be conducted on conscious animals.

CHAPTER III

MATERIALS - METHODS

Ten mongrel dogs, of either sex (4 males, 6 females) and varying body weights (average 16 ± 2 S.E. kilograms) were obtained from the Oklahoma State University animal care unit. Upon arrival, and once per day for three consecutive days, direct blood smear examinations for heartworms, complete blood counts and serum electrolytes were determined for each animal. A lead II electrocardiogram was obtained to screen for any cardiac abnormalities.

Each dog was vaccinated for canine distemper and rabies at the time of arrival. All dogs were housed singly in runs and fed standard laboratory chow and water ad libitum.

Preparation of Animals

One week prior to experimentation, each dog was anesthetized with thiopental sodium[a] (3mg/kg) and methoxyflurane.[b] The left carotid artery and left jugular vein were cannulated with a combination polyethylene[c]/Silastic tubing[d] cathether. The catheters were filled with heparinized saline[e], sutured in place, and externally secured with gauze bandaging and Elastikon. The dogs were allowed free access to food and water. Following surgery,

catheters were flushed daily with 1000 U sodium heparin in 1 ml sterile saline.[f]

Two days prior to experimentation, body fluid volumes were determined using antipyrine[g] for total body (20 mg/kg); potassium thiocyanate[h] for total extracellular fluid (15 mg/kg); and Evan's blue[i] for plasma and blood volume (0.4 mg/kg). At each sampling interval, the indices which were to be measured during the experiment were also determined. These indices were: serum sodium, potassium chloride; arterial pH, PCO_2, PO_2, HCO_3^-, base excess; red blood cell count, hemoglobin concentration, packed cell volume, total plasma protein and anion gap. The sampling intervals were at time 0 (pre-dye injection), +10,20,30 and 90 minutes.

Experimental Procedure

The basic experimental plan consisted of bleeding each animal 20% of their measured blood volume over a 15-30 minute interval, following a 20 minute stabilization period. Blood samples were drawn at intervals, for the above indices, and immediate blood gas analysis, until acidosis was established. Criteria for an acidotic state was a base excess of $\geq$ -4 mmol/l. After the establishment of a metabolic acidosis, one of five treatments was initiated.

Table III is a list of treatments used in this experiment. The blood sampling intervals were at time 0 (immediately after cessation of treatment), +10, 20, 20, 90, 150, 210 and 1440

TABLE III

LIST OF TREATMENTS

TREATMENT #	
1	Control Group - no fluid replacement
2	Fluid Replacement with:
	100 mEq/l NaCl
	25 mEq/l sodium lactate
	25 mEq/l sodium gluconate
3	Fluid Replacement with:
	100 mEq/l NaCl
	25 mEq/l sodium lactate + correction
	25 mEq/l sodium gluconate - correction
4	Fluid Replacement with:
	100 mEq/l NaCl
	25 mEq/l $NaHCO_3$
	25 mEq/l sodium gluconate
5	Fluid Replacement with:
	100 mEq/l NaCl
	25 mEq/l $NaHCO_3$ + correction
	25 mEq/l sodium gluconate - correction

Fluid volume replaced was equal to 20% of the measured blood volume. The correction was the quantity, in mEq/l, that was equal to 0.3 x Body Wt (Kg) x Base Excess.

minutes. The dye indicators for determination of body water distribution were injected after the zero time sample. Each dog went through two experimental trials separated by a one week interval, and each treatment was assigned four dogs.

Sample Analysis

Arterial blood gases and pH were measured on a Corning 175 automatic pH/blood gas system. Serum sodium and potassium were measured with a Beckman Klina Flame^R; serum Chloride with a Digital chloridometer, Buckler Instruments, Fort Lee, NJ. All electrolyte samples were assayed by the Clinical Pathology Laboratory, Oklahoma State University Veterinary Teaching Hospital. Red blood cell counts were made with a Unopette^R system; hematocrit with microhematocrit methods; hemoglobin concentration with a Spencer hemoglobinometer; total protein with a refractometer.

Chemical procedures for dye determination are in the appendix. Samples were analyzed with a Beckman DU-8 spectrophotometer.

Statistical Analysis

The experimental design was an incomplete block design. The block design is an experimental plan where the block may be an individual or group of individuals that share some characteristic which may influence the response to the treatment. In a complete block design, all treatments are associated with each

block. However, in the incomplete block design not all treatments are associated with each block. In this study, the blocks were the individual dogs and two treatments were randomly assigned to each dog (Figure 4). This design was also set up to be a balanced design. The design was balanced with respect to the fact that 1) each treatment was assigned four dogs, and 2) each treatment appeared twice per trial (see Figure 4). The original design called for 2 trials per dog which would yield 20 total trials. However, technical problems resulted in the loss of data from 3 trials, leaving an unbalanced design.

The design used in this study also contained elements of a split unit design. In a split unit design, the investigator is usually interested in at least two factors, one factor contained within the other (i.e., nested design). The feature of this design is that there is a main unit or factor which is randomized within the block and there is a subunit which is randomized within the main unit. In this study, the main unit is each dog during a trial and the subunits are the time intervals when each observation was made within each trial. The main unit measures the effect of a treatment on a dog during a trial. The subunit measures the effect of time on each treatment within each trial.

In this design, the accuracy of the evaluation on the main unit (treatment effect) is usually less than that of the subunit (time effect) for two reasons: 1) the error term of the main unit is associated with fewer degrees of freedom, therefore, the F statistic needed to demonstrate significant effect needs to be

TREATMENT:	1	2	3	4	5
Dog #					
1				*	*
2		*	*		
3			*	*'	
4	*	*			
5		*			*
6	*		*		
7			*		*'
8		*		*	
9	*'			*	
10	*				*

Figure 4. Experimental Design

* Indicates treatment each dog received.

' Indicates missing values for that treatment

large; and 2) the main unit effect is averaged over time, so any variability over time is included in the error term for treatment effect (main unit error). It should be noted that, due to the above reasons, a nonsignificant result may indicate the experiment was not precise enough to detect any differences. On the other hand, a significant result would be strong evidence in favor of that effect.

The data was analyzed two times by analysis of variance procedures using the General Linear Models (GLM) program of the Statistical Analysis System (SAS) on the Oklahoma State University IBM 360 computer. The first analysis divided the data into four categories: 1) treatment effect; 2) dog effect; 3) time effect, and 4) time x treatment interaction.

The effect of treatment was averaged over time, beginning with time 0 or the sample immediately following cessation of the thirty minute treatment period. The test for dog effect measured the variability between dogs against the variability within dogs (main unit error term). The variability within dogs is calculated as the variability between trials for each dog. The effect of time and time x treatment interaction were then measured. If a significant result appeared for the time x treatment interaction, this was taken to mean the magnitude of the response to one treatment was not parallel to that seen in other treatments over time.

If the test for the variability between dogs was not significant ($p>.05$), the degrees of freedom and sum of squares for dogs were pooled with the main unit error, thus increasing the

ability to detect treatment differences. It also simplified the calculation of standard errors to be used in comparing treatment differences in this unbalanced design. Thus, the second analysis utilized the pooled sum of squares for the main unit error and the treatment effect was tested using this new term. The time and time x treatment interaction were not affected and thus the result from the second analysis of variance was the same as from the first analysis. Table IV gives examples of the two analysis of variance tables and the associated degrees of freedom.

In this study, for all the variables studied, there was no significant difference between dogs. Therefore, all results reported are from the second analysis. However, the results of the second analysis were similar to those of the first analysis with respect to treatment effect, time effect and time x treatment interaction.

If the treatment effect was significant ($p<.05$) comparisons of groups of treatments and individuals were made. Referring to Table III (list of treatments) the comparisons of treatments were as follows:

treatment(s)	vs.	treatment(s)
4		5
4,5		1,2,3
1		2,3
2		3
4		1
4		2
4		3
5		1
5		2
5		3

TABLE IV

SAMPLE ANALYSIS OF VARIANCE TABLES

a. Table used for determination of differences between dogs

Source	Degrees of Freedom
Treatment Effect	4
Dog Effect	9
Main Unit Error	3
Time Effect	7
Time x Treatment Interaction	28
Sub Unit Error	79

b. Table used with the pooled data:

Source	Degrees of Freedom
Treatment Effect	4
Main Unit Error	12
Time Effect	7
Time x Treatment Interaction	28
Subunit Error	79

The first comparison was a test for the difference between the two bicarbonate solutions; the second was a test comparing the bicarbonate solutions against the lactate solutions and the control; the third was a test of the difference of the control against the lactate solutions and the fourth was a test of the difference between the two lactate solutions. The remainder of the comparisons were individual tests of the bicarbonate solutions (treatments 4 or 5) against the remaining treatments (1, 2, or 3).

Standard errors were calculated in the following manner:

$$\text{Std error} = \sqrt{\frac{\text{variance of main unit error} + \text{variance of subunit error}}{N}}$$

where N is the number of observations for that particular time and treatment.

The changes in body fluid distribution were analyzed with a paired t-test, comparing the values obtained two days prior to the experiment with the values obtained immediately following treatment.

CHAPTER IV

RESULTS

Results of this study will be presented using the treatment numbers. For a description for each treatment, refer to Table III.

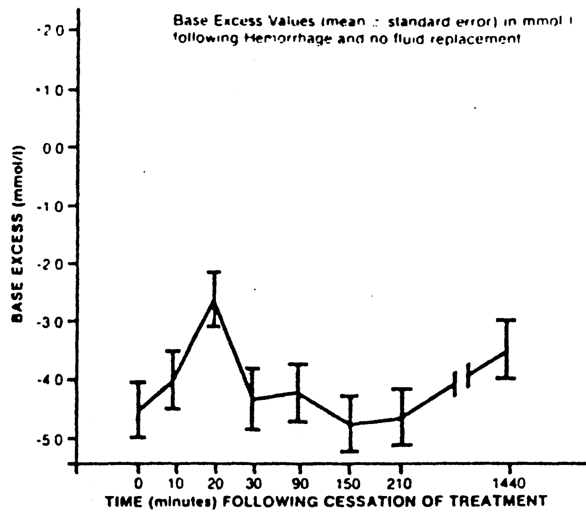
Base Excess

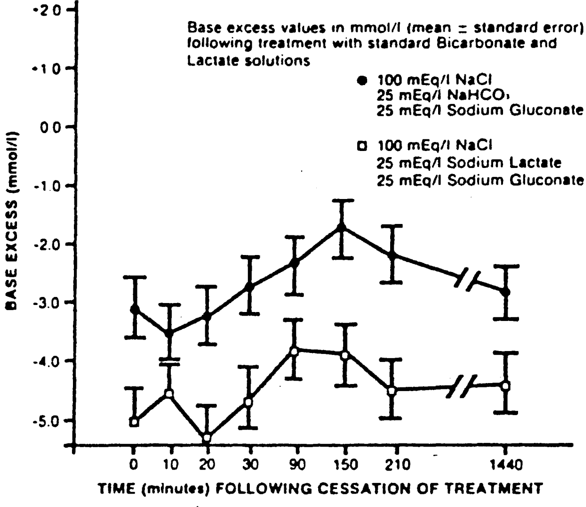
Table V and Figure 5 show the mean changes in base excess (± standard errors) for each treatment. In terms of correcting the acidosis, based on changes in base excess, it can be seen in Figure 5 that the group receiving the standard bicarbonate solution plus the dose correction for the base excess (treatment 5) showed the best response. Starting at the base excess of -4mEq/l, immediately after treatment, the base excess went to +0.16, then remained well within the normal range for the rest of the observation period. Treatment number 4 (the standard bicarbonate solution) also proved effective in correcting the base excess. This is in contrast to the lactate solutions (treatments 2 and 3) both of which did not change the base excess significantly from the initial pretreatment level of -4.0. This pattern follows the results from the comparison tests which showed: 1) treatments 4 and 5 were significantly different from treatments 1, 2, and 3

TABLE V

BASE EXCESS VALUES FOR ALL TREATMENT GROUPS (mmol/l)

TREATMENT	TIME (MINUTES) POST TREATMENT	N	MEAN	STANDARD ERROR
1 Control - no fluid replacement	0	3	-4.4	.578
	10	3	-3.9	.578
	20	3	-2.5	.578
	30	3	-4.2	.578
	90	3	-4.1	.578
	150	3	-4.6	.578
	210	3	-4.7	.578
	1440	3	-3.3	.578
2 100 mEq/l NaCl, 25 mEq/l sodium lactate, 25 mEq/l sodium gluconate	0	4	-4.7	.501
	10	4	-4.3	.501
	20	4	-5.1	.501
	30	4	-4.4	.501
	90	4	-4.4	.501
	150	4	-3.9	.501
	210	4	-4.5	.501
	1440	4	-4.4	.501
3 100 mEq/l NaCl, 25 mEq/l sodium lactate + Base Excess correction, 25 mEq/l sodium gluconate - Base Excess correction	0	4	-3.1	.501
	10	4	-3.9	.501
	20	4	-4.2	.501
	30	4	-4.6	.501
	90	3	-3.9	.578
	150	4	-3.9	.578
	210	4	-4.3	.501
	1440	4	-4.1	.501
4 100 mEq/l NaCl, 25 mEq/l $NaHCO_3$, 25 mEq/l sodium gluconate	0	3	-3.1	.578
	10	3	-3.1	.578
	20	3	-2.4	.578
	30	3	-2.7	.578
	90	2	-2.3	.708
	150	2	-1.7	.708
	210	2	-2.2	.708
	1440	3	-2.8	.578
5 100 mEq/l NaCl, 25 mEq/l $NaHCO_3$ + Base Excess correction, 25 mEq/l sodium gluconate - Base Excess correction	0	3	0.16	.578
	10	3	-1.0	.578
	20	3	-1.5	.578
	30	3	-1.4	.578
	90	3	-1.0	.578
	150	3	-1.3	.578
	210	3	-1.5	.578
	1440	3	-0.9	.578





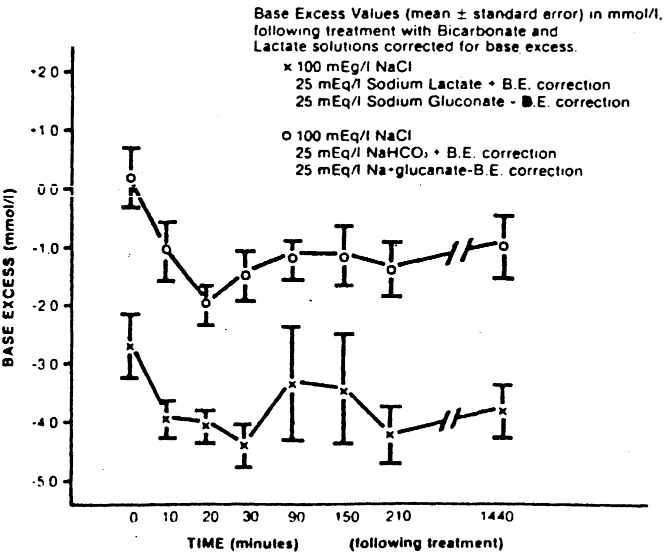


Figure 5. Mean Changes in Base Excess
vs. Time For Each Treatment

when compared as a group (e.g., 4, 5 vs. 1, 2, 3) or when compared individually (e.g., 4 vs. 1); 2) neither treatment 2 nor 3 were significantly different from the control (treatment 1); and 3) treatment 4 was significantly different from treatment 5.

Bicarbonate Concentration

Table VI and Figure 6 show the mean changes in bicarbonate concentration (± standard errors). Referring to the graphs of the changes in bicarbonate concentration, it can be seen that, for all treatment groups, the values were below normal and were close to 30 mEq/l for treatments 4 and 5, and approximately 17-18 mEq/l for treatments 1, 2 and 3. The comparisons between treatment groups showed a similar pattern to that for the variable base excess: treatments 4 and 5 (bicarbonate treatments) were significantly different from the control (treatment 1) and treatments 2 and 3 (lactate treatments); and treatments 2 and 3 were not significantly different from the control. There was, however, no difference between treatments 4 and 5.

PCO_2

Table VII and Figure 7 show the mean changes in PCO_2. It can be seen that, for all treatment groups, the values were below normal. Comparisons between treatment groups showed: significant differences between the bicarbonate solutions and the other treatment groups; and no significant differences between the lactate groups and the control.

TABLE VI

BICARBONATE CONCENTRATION (mEq/l)

TREATMENT	TIME(MINUTES) POST TREATMENT	N	MEAN	STANDARD ERROR
1 Control - no fluid replacement	0	3	17.1	.77
	10	3	18.0	.77
	20	3	19.6	.77
	30	3	17.3	.77
	90	3	17.9	.77
	150	3	16.9	.77
	210	3	17.0	.77
	1440	3	18.9	.77
2 100 mEq/l sodium chloride 25 mEq/l sodium lactate 25 mEq/l sodium gluconate	0	4	17.5	.67
	10	4	18.1	.67
	20	4	17.0	.67
	30	4	18.0	.67
	90	4	18.0	.67
	150	4	19.1	.67
	210	4	17.8	.67
	1440	4	17.8	.67
3 100 mEq/l sodium chloride 25 mEq/l sodium lactate + Base Excess correction 25 mEq/l sodium gluconate - Base Excess correction	0	4	19.2	.67
	10	4	18.3	.67
	20	4	17.5	.67
	30	4	17.1	.67
	90	3	17.8	.77
	150	4	18.3	.67
	210	4	17.8	.67
	1440	4	18.0	.67
4 100 mEq/l sodium chloride 25 mEq/l sodium bicarbonate 25 mEq/l sodium gluconate	0	3	19.3	.77
	10	3	20.6	.77
	20	3	19.7	.77
	30	2	20.2	.94
	90	2	20.9	.94
	150	2	21.4	.94
	210	2	20.7	.94
	1440	3	19.3	.77
5 100 mEq/l sodium chloride 25 mEq/l sodium bicarbonate + Base Excess correction 25 mEq/l sodium gluconate -Base Excess correction	0	3	22.6	.77
	10	3	21.7	.77
	20	3	20.9	.77
	30	3	21.2	.77
	90	3	21.8	.77
	150	3	21.3	.77
	210	3	21.0	.77
	1440	3	21.4	.77

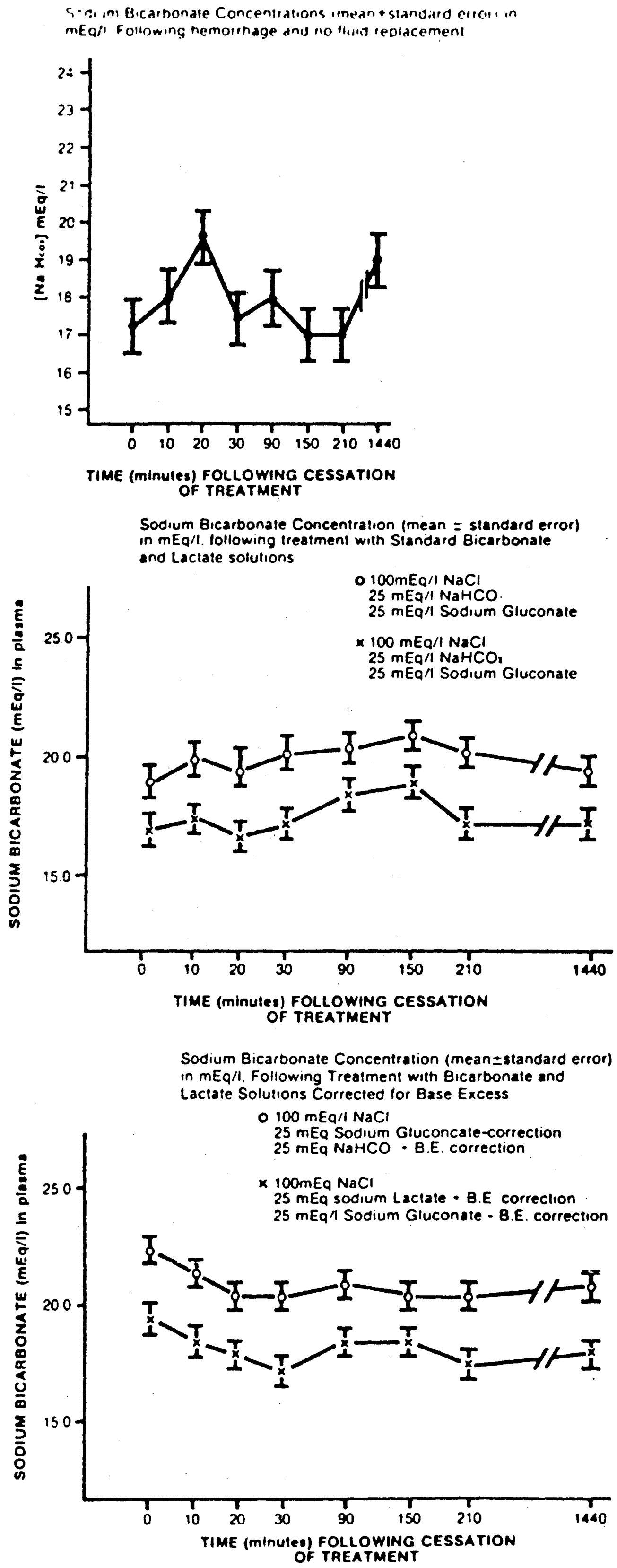
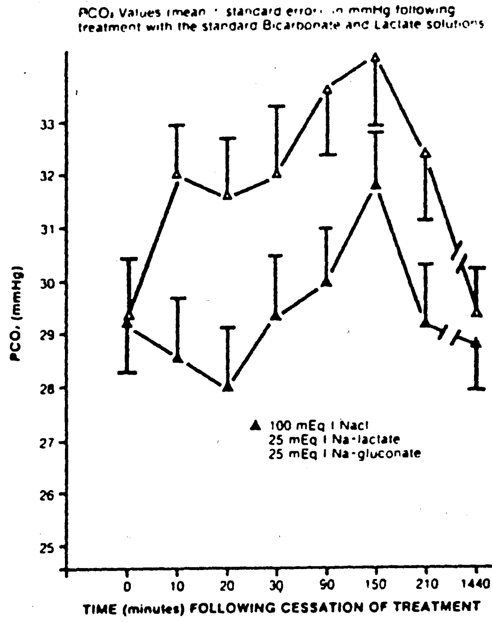


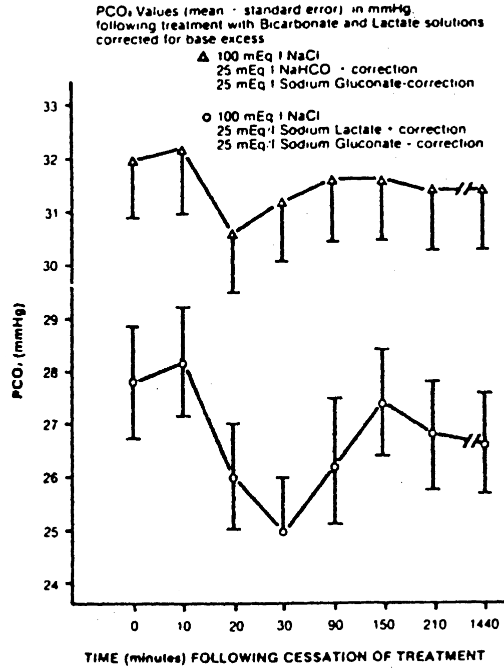
Figure 6. Bicarbonate Concentrations Vs. Time Following Cessation Of Treatment For All Treatment Groups

TABLE VII

PCO_2 VALUES FOR ALL TREATMENT GROUPS (mm Hg)

TREATMENT	TIME(MINUTES) POST TREATMENT	N	MEAN	STANDARD ERROR
1 Control	0	3	26.2	1.64
no fluid replacement	10	3	28.0	1.64
	20	3	29.4	1.64
	30	3	26.4	1.64
	90	3	27.2	1.64
	150	3	25.1	1.64
	210	3	26.0	1.64
	1440	3	29.0	1.64
2 100 mEq/l NaCl	0	4	29.1	1.42
25 mEq/l sodium lactate	10	4	28.5	1.42
25 mEq/l sodium gluconate	20	4	28.0	1.42
	30	4	29.4	1.42
	90	4	30.0	1.42
	150	4	31.7	1.42
	210	4	29.2	1.42
	1440	4	28.7	1.42
3 100 mEq/l NaCl	0	4	28.7	1.42
25 mEq/l sodium lactate +	10	4	29.2	1.42
Base Excess correction	20	4	26.9	1.42
25 mEq/l sodium gluconate -	30	4	25.9	1.42
Base Excess correction	90	3	27.2	1.64
	150	4	28.2	1.42
	210	4	27.8	1.42
	1440	4	27.6	1.42
4 100 mEq/l NaCl	0	3	29.4	1.64
25 mEq/l $NaHCO_3$	10	3	32.0	1.64
25 mEq/l sodium gluconate	20	3	31.5	1.64
	30	2	32.0	2.01
	90	2	33.6	2.01
	150	2	34.2	2.01
	210	2	32.3	2.01
	1440	3	29.3	1.64
5 100 mEq/l NaCl	0	3	33.0	1.64
25 mEq/l $NaHCO_3$ + Base Excess correction	10	3	33.1	1.64
	20	3	31.6	1.64
25 mEq/l sodium gluconate -	30	3	32.1	1.64
Base Excess correction	90	3	32.5	1.64
	150	3	32.6	1.64
	210	3	32.3	1.64
	1440	3	32.4	1.64





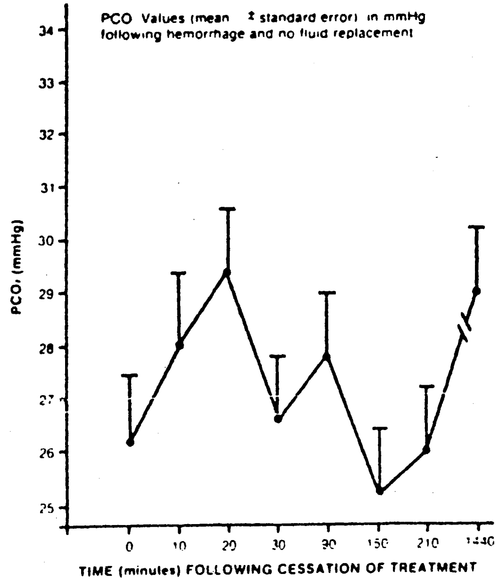


Figure 7. Changes in PCO_2

Anion Gap

Referring to the table of means (Table VIII), it can be seen that, although all treatment groups showed greater than normal anion gaps, the groups receiving the bicarbonate solutions (treatments 4 and 5) showed smaller anion gaps than the other groups. There was no significant difference between any of the treatment groups ($p>.05$).

Packed Cell Volume

Referring to Table IX, it can be seen that the packed cell volume (PCV) decreased over time and showed similar changes in treatment groups 1-4. This would account for a significant time effect ($p<.05$), whereas there was not a statistically significant treatment difference.

Na^+, Cl^-, K+

Tables X, XI, and XII show the mean values (in mEq/l) for these ions. It can be seen that the values for sodium and chloride were relatively constant over time, while there was a slight increase in potassium concentration in all treatment groups. Serum chloride values were slightly higher than normal for treatment groups 1 and 4. There was no significant difference, however, between treatments and control of these variables.

pH

There was no significant treatment or time effect for this

TABLE VIII

ANION GAP VALUES FOR ALL TREATMENT GROUPS (mEq/l)

TREATMENT	TIME (MINUTES) POST TREATMENT	N	MEAN	STANDARD ERROR
1 Control - no fluid replacement	0	3	20.93	3.72
	10	3	28.13	3.72
	20	3	24.26	3.72
	30	3	27.16	3.72
	90	3	27.23	3.72
	150	3	27.96	3.72
	210	3	30.83	3.72
	1440	3	25.3	3.72
2 100 mEq/l NaCl 25 mEq/l sodium lactate 25 mEq/l sodium gluconate	0	4	28.9	3.22
	10	4	26.1	3.22
	20	4	26.9	3.22
	30	4	30.5	3.22
	90	4	28.5	3.22
	150	4	27.8	3.22
	210	4	28.8	3.22
	1440	4	29.0	3.22
3 100 mEq/l NaCl 25 mEq/l sodium lactate + Base Excess correction 25 mEq/l sodium gluconate - Base Excess correction	0	4	30.5	3.22
	10	4	31.2	3.22
	20	4	28.5	3.22
	30	4	30.6	3.22
	90	3	31.6	3.72
	150	4	31.0	3.22
	210	4	30.4	3.22
	1440	4	29.8	3.22
4 100 mEq/l NaCl 25 mEq/l $NaHCO_3$ 25 mEq/l sodium gluconate	0	3	21.3	3.72
	10	3	22.8	3.72
	20	3	20.5	3.72
	30	2	22.4	4.56
	90	2	20.9	4.56
	150	2	23.85	4.56
	210	2	28.4	4.56
	1440	3	22.1	3.72
5 100 mEq/l NaCl 25 mEq/l $NaHCO_3$ + Base Excess correction 25 mEq/l sodium gluconate - Base Excess correction	0	3	26.2	3.72
	10	3	21.9	3.72
	20	3	24.0	3.72
	30	3	22.1	3.72
	90	3	24.0	3.72
	150	3	24.6	3.72
	210	3	28.5	3.72
	1440	3	25.9	3.72

TABLE IX

PACKED CELL VOLUME

TREATMENT	TIME(MINUTES) POST TREATMENT	N	MEAN	STANDARD ERROR
1 Control - no fluid replacement	0	3	38.3	2.56
	10	3	35.3	2.56
	20	3	35.0	2.56
	30	3	35.0	2.56
	90	3	33.6	2.56
	150	3	34.0	2.56
	210	3	34.0	2.56
	1440	3	34.0	2.56
2 100 meq/l NaCl 25 mEq/l sodium lactate 25 mEq/l sodium gluconate	0	4	34.7	1.42
	10	4	33.6	1.42
	20	4	33.7	1.42
	30	4	33.7	1.42
	90	4	33.2	1.42
	150	4	32.7	1.42
	210	4	32.7	1.42
	1440	4	33.5	1.42
3 100 mEq/l NaCl 25 mEq/l sodium lactate + Base Excess correction 25 mEq/l sodium gluconate - Base Excess correction	0	4	34.7	1.42
	10	4	34.5	1.42
	20	4	34.0	1.42
	30	4	33.7	1.42
	90	3	32.6	2.56
	150	4	33.7	1.42
	210	4	33.0	1.42
	1440	4	32.0	1.42
4 100 mEq/l NaCl 25 mEq/l $NaHCO_3$ 25 mEq/l sodium gluconate	0	3	32.3	2.56
	10	3	31.6	2.56
	20	3	30.3	2.56
	30	2	32.5	2.56
	90	2	33.0	3.13
	150	2	31.5	3.13
	210	2	31.5	3.13
	1440	3	29.3	2.56
5 100 mEq/l NaCl 25 mEq/l $NaHCO_3$ + Base Excess correction 25 mEq/l sodium gluconate - Base Excess correction	0	3	32.6	2.56
	10	3	32.6	2.56
	20	3	32.3	2.56
	30	3	33.0	2.56
	90	3	32.0	2.56
	150	3	33.3	2.56
	210	3	32.6	2.56
	1440	3	32.3	2.56

TABLE X

SERUM SODIUM CONCENTRATIONS (mEq/1)

TREATMENT	TIME(MINUTES) POST TREATMENT	N	MEAN	STANDARD ERROR
1 Control -	0	3	148.6	1.73
no fluid replacement	10	3	151.6	1.73
	20	3	150.3	1.73
	30	3	152.3	1.73
	90	3	151.6	1.73
	150	3	151.0	1.73
	210	3	153.3	1.73
	1440	3	151.3	1.73
2 100 mEq/1 NaCl	0	4	148.5	1.50
25 mEq/1 sodium lactate	10	4	149.2	1.50
25 mEq/1 sodium gluconate	20	4	148.0	1.50
	30	4	149.7	1.50
	90	4	147.2	1.50
	150	4	147.7	1.50
	210	4	147.5	1.50
	1440	4	148.0	1.50
3 100 mEq/1 NaCl	0	4	148.0	1.50
25 mEq/1 sodium lactate	10	4	149.0	1.50
+ Base Excess correction	20	4	148.0	1.50
25 mEq/1 sodium gluconate	30	4	147.5	1.50
- Base Excess correction	90	3	146.0	1.73
	150	4	145.7	1.50
	210	4	146.7	1.50
	1440	4	146.2	1.50
4 100 mEq/1 NaCl	0	3	152.6	1.73
25 mEq/1 $NaHCO_3$	10	3	152.3	1.73
25 mEq/1 sodium gluconate	20	3	151.6	1.73
	30	2	151.5	1.73
	90	2	152.0	2.12
	150	2	153.0	2.12
	210	2	153.5	2.12
	1440	3	152.0	1.73
5 100 mEq/1 NaCl	0	3	150.0	1.73
25 mEq/1 $NaHCO_3$ + Base Excess Correction	10	3	148.6	1.73
	20	3	150.6	1.73
25 mEq/1 sodium gluconate	30	3	152.6	1.73
- Base Excess correction	90	3	151.0	1.73
	150	3	151.6	1.73
	210	[illegible]	[illegible]	[illegible]

TABLE XI

SERUM CHLORIDE CONCENTRATIONS (mEq/l)

TREATMENT	TIME (MINUTES) POST TREATMENT	N	MEAN	STANDARD ERROR
1 Control - no fluid replacement	0	3	114.6	3.40
	10	3	109.6	3.40
	20	3	110.6	3.40
	30	3	112.0	3.40
	90	3	110.6	3.40
	150	3	110.3	3.40
	210	3	109.6	3.40
	1440	3	111.3	3.40
2 100 mEq/l NaCl 25 mEq/l sodium lactate 25 mEq/l sodium gluconate	0	4	106.0	2.95
	10	4	109.0	2.95
	20	4	107.5	2.95
	30	4	105.2	2.95
	90	4	104.7	2.95
	150	4	104.7	2.95
	210	4	105.0	2.95
	1440	4	105.2	2.95
3 100 mEq/l NaCl 25 mEq/l sodium lactate +Base Excess correction 25 mEq/l sodium gluconate - Base Excess correction	0	4	102.0	2.95
	10	4	103.2	2.95
	20	4	106.0	2.95
	30	4	103.7	2.95
	90	3	101.3	3.40
	150	4	100.5	2.95
	210	4	102.5	2.95
	1440	4	102.7	2.95
4 100 mEq/l NaCl 25 mEq/l $NaHCO_3$ 25 mEq/l sodium gluconate	0	3	116.0	3.40
	10	3	113.0	3.40
	20	3	115.6	3.40
	30	2	113.0	4.17
	90	2	114.5	4.17
	150	2	112.0	4.17
	210	2	108.5	4.17
	1440	3	114.6	3.40
5 100 mEq/l NaCl 25 mEq/l $NaHCO_3$ + Base Excess correction 25 mEq/l sodium gluconate - Base Excess correction	0	3	105.3	3.40
	10	3	109.0	3.40
	20	3	109.6	3.40
	30	3	113.3	3.40
	90	3	109.3	3.40
	150	3	109.6	3.40
	[illegible]	[illegible]	[illegible]	[illegible]

TABLE XII

SERUM POTASSIUM CONCENTRATIONS (mEq/l)

TREATMENT	TIME (MINUTES) POST TREATMENT	N	MEAN	STANDARD ERROR
1 Control - no fluid replacement	0	3	4.13	.232
	10	3	4.20	.232
	20	3	4.23	.232
	30	3	4.20	.232
	90	3	4.13	.232
	150	3	4.23	.232
	210	3	4.23	.232
	1440	3	4.20	.232
2 100 mEq/l NaCl	0	4	4.02	.201
25 mEq/l sodium lactate	10	4	4.00	.201
25 mEq/l sodium gluconate	20	4	4.05	.201
	30	4	4.07	.201
	90	4	4.05	.201
	150	4	4.05	.201
	210	4	4.20	.201
	1440	4	4.15	.201
3 100 mEq/l NaCl	0	4	3.77	.201
25 mEq/l sodium lactate	10	4	3.87	.201
+ Base Excess correction	20	4	4.07	.201
25 mEq/l sodium gluconate	30	4	4.02	.201
- Base Excess correction	90	3	4.26	.232
	150	4	4.10	.201
	210	4	4.07	.201
	1440	4	4.32	.201
4 100 mEq/l NaCl	0	3	4.00	.232
25 mEq/l $NaHCO_3$	10	3	4.13	.232
25 mEq/l sodium gluconate	20	3	4.20	.232
	30	2	4.20	.284
	90	2	4.35	.284
	150	2	4.25	.284
	210	2	4.15	.284
	1440	3	4.10	.232
5 100 mEq/l NaCl	0	3	4.23	.232
25 mEq/l $NaHCO_3$ + Base	10	3	3.93	.232
Excess correction	20	3	4.00	.232
25 mEq/l sodium gluconate	30	3	4.06	.232
- Base Excess correction	90	3	4.13	.232
	150	3	4.00	.232
	210	3	4.16	.232

variable and all values were within normal limits.

Dye Determinations

There was no significant difference in the pre-treatment and post-treatment values for any of the body fluid spaces for treatments 2-5 (Table XIII). There was a significant difference in the pre-treatment/post-treatment values for blood volume in the control group (treatment 1). The values indicate a reduction in blood volume of approximately 17% following hemorrhage.

TABLE XIII

TABLE OF MEAN ± STANDARD ERRORS FOR CHANGES IN FLUID COMPARTMENTS, PRECEDING AND AFTER TREATMENT (VALUES IN LITERS)

		PRETREATMENT	POST TREATMENT
Blood Volume	TRT#		
	1	1.37, .219	1.14, .19*
	2	1.35, .111	1.39, .181
	3	1.11, .164	1.06, .14
	4	1.00, .409	.98, .37
	5	.94, .233	.933, .218
ECF	1	3.67, .58	3.39, .563
	2	3.7, .34	3.79, .36
	3	3.02, .50	3.06, .49
	4	2.61, 1.0	2.61, .98
	5	2.4, .572	2.4, .53
ISF	1	2.7, .44	2.6, .43
	2	2.8, .28	2.84, .26
	3	1.9, .372	2.3, .4
	4	1.9, .78	1.9, .73
	5	1.8, .42	1.78, .39
TBW	1	11.18, 1.6	10.9, 1.6
	2	11.6, 1.2	11.7, 1.1
	3	8.7, 1.5	8.7, 1.3
	4	7.8, 3.0	7.8, 3.0
	5	7.6, 1.6	7.5, 1.6

CHAPTER V

DISCUSSION

The changes that occur in the cardiovascular system following the loss of blood, are designed to maintain the blood pressure. Through the release of norepinephrine and epinephrine, vasoconstriction occurs, along with an increase in blood pressure. Vasoconstriction leads to a decreased perfusion of certain tissues, to supply blood to vital organs. The shift of blood is usually away from organs such as the gastrointestinal tract, to maintain perfusion to the heart, lungs and brain. This decrease in perfusion to the GI tract will lead to an increased dependence on local anaerobic metabolism.

The changes in cellular metabolism during anaerobiosis include an increased dependence on glycolysis for energy.[50] This is due to inhibition of the high energy yielding reactions of the tricarboxylic acid cycle. The result is an increase in the local concentrations of hydrogen ions, adenosine, lactic acid and other organic acids and carbon dioxide.

Diagnosis of Metabolic Acidosis

The changes that occur in the pH, the bicarbonate buffer system and PCO_2 can be monitored with blood gas analysis. In the

case of a simple metabolic acidosis (i.e., no other acid base disturbance is present) a diagnosis can be established by the presence of a low plasma bicarbonate, low blood pH and reduced PCO_2.

The decrease in plasma sodium bicarbonate is a result of titration with the organic acids. The subsequent decrease in pH and further increase in hydrogen ion (or organic acid) concentration will lead to stimulation of respiratory ventilation. The increased ventilation in response to a decrease in pH will lead to a decrease in PCO_2 in an attempt to restore pH. Depending on the relative magnitude of the changes in PCO_2 and sodium bicarbonate concentration, the pH will change accordingly.

An index used in estimating the severity of acid base disturbances is the base excess. The base excess is the deviation in bicarbonate concentration from the normal value of 24 mEq/l. It is calculated from the bicarbonate concentration, hemoglobin and pH. It is a reflection of the titration of blood and thus more accurate than just the bicarbonate concentration or pH in describing changes in the bicarbonate buffer system. Acidosis is associated with a negative base excess; alkalosis is associated with a positive excess.

In this study, a base excess of $\geq$ -4 mmol/l was the criterion for metabolic acidosis and treatment. The results from the control group indicate that removal of 20% of the measured blood volume of conscious dogs is sufficient to lower the pH and increase the base excess. It can also be seen that, even after 24

hours, the animals are still acidotic by the criteria of the experiment. The sodium bicarbonate concentrations remain low, as well, throughout the experiment. The changes in PCO_2 seen in this group were also expected, and are probably a result of the respiratory compensation for metabolic acidosis. Although the respiratory rates were not measured in all dogs, respiration rate and depth were noted for animals of the control group following hemorrhage. In these animals, rate and depth increased following hemorrhage.

Another measure of the severity of metabolic acidosis is the anion gap. Data from the control group indicate the presence of a clinical acidosis (anion gap > 20 mEq/l), and this was observed through all measured intervals. The increase in the anion gap is a result of an increase in organic acid anions (i.e., lactic) which are known to increase as a result of tissue hypoxia produced by vasoconstriction following blood loss. Although the actual composition of the increase in unmeasured anions was not determined, Huckabee showed that a loss of blood will produce an increase in the concentration of lactic acid. This increase is seen even when blood loss is not sufficient to produce noticeable changes in blood pressure.

The importance of changes in the above indices (PCO_2, anion gap, bicarbonate concentration and base excess) is best seen in those groups receiving fluid replacement. In this study, two levels of base concentration were used. The lower level represented a standard or maintenance dose. The intention was to add

a concentration of base that, when infused, would not result in a dilutional acidosis and this dose was 25 mEq/l. The objective of the higher dose was to add base in a contration to correct for the observed base excess. The two bases utilized were sodium lactate and sodium bicarbonate in a solution containing sodium chloride and sodium gluconate.*

Dilutional Acidosis

The data from the two groups receiving the low dose of base seem to indicate that, while the sodium bicarbonate solution restored the base excess to within normal limits (0 ± 3 mmol/l), there was still some apparent dilution of the bicarbonate buffer system. However, this may be due to the fact that there was not enough base present in the infusion solution to correct for the sodium bicarbonate already depleted. This will be discussed in more detail below.

The group receiving the lower dose of sodium lactate did not respond any differently than the control. There are a few possibilities for this result. First, although sodium lactate can act as a base and accept hydrogen ions at pH values near its pKa (3.9), at a pH of 7.4 the ionization constant favors the formation of sodium lactate and not lactic acid. At a pH of 7.4, 99% of the lactate present exists as the sodium salt.

Second, if the organic acid acidosis was attributable to an increase in lactic acid, any titration reaction would be:

---

*NOTE: It has been shown that sodium gluconate has no observable effect on any of the indices measured.

sodium lactate + lactic acid   lactic acid + sodium lactate

In other words, there would be no net decrease in the concentration of lactic acid.

Another factor in the lack of response of the low dose lactate group is that the metabolic pathways of lactate utilization are inhibited during acidosis. The rationale behind the use of lactate as an alkalinizing agent is that lactate is metabolized into bicarbonate. However, the oxidation of lactate will only produce CO_2 and H_2O via the TCA cycle. If this carbon dioxide and water would combine to form carbonic acid, which would then dissociate into hydrogen and bicarbonate ions, the bicarbonate ion could combine with the sodium ion from the sodium lactate. The hydrogen ion would then have to be buffered, and it would appear to defeat the purpose of adding the base to correct the acidosis. Another problem with this idea is the fate of the sodium ion from the infused sodium lactate. Once the lactate ion is released, the sodium ion would either have to be eliminated (via the kidney) or it would have to combine with another anion, such as intracellular chloride or bicarbonate. This gives yet another possible explanation for the rise in bicarbonate observed in this study at 90-150 minutes and in other investigations: ion exchange. This ion exchange would be extracellular lactate for intracellular bicarbonate or chloride. This might account for the higher than normal values obtained for serum chloride concentration. The magnitude of the change in plasma

sodium bicarbonate in the present study was not as great as that observed by Hartsfield et al.[99] It should be noted, however, that, in the present study, the concentration of lactate used was much smaller (25 mEq/l in a volume equal to 20% of the blood volume) than than in the Hartsfield study (6.6 mEq/kg).

The use of the higher doses of these bases was intended to correct for the base excess. The group receiving the high dose of sodium lactate did not respond any differently than the control or low dose of lactate. The group of animals receiving the corrected dose of sodium bicarbonate had the best response, with respect to changes in the base excess. It should be noted that, even in this group, the bicarbonate values were still lower than normal. This might be explained by the fact that the correction factor, 0.3 x Body Wt (Kg), represents a change in the bicarbonate concentration of only extracellular fluid. It has been shown that 50% of the buffering of acid occurs with intracellular buffers. Therefore, due to the dampening of changes of extracellular bicarbonate by hemoglobin, the intracellular stores of $NaHCO_3$ are being depleted before any observable changes in extracellular $NaHCO_3$ occur. This correction factor (0.3) thus underestimates the depletion of the bicarbonate buffer due to consumption by organic acid. This is consistent with the observations of Garella et al,[42] who have shown that the volume of distribution of sodium bicarbonate increases with the severity of the acidosis.

The changes in the concentration of the electrolytes, sodium

and potassium, are consistent with changes that might be seen during organic acid acidosis. The changes in sodium are approximately 1% from time zero, which is a similar percentage to that obtained by Swan and Pitts. This corresponds to buffering by plasma proteins. The changes in plasma K^+ are consistent with the entry of organic acids into the intracellular fluid. If the acid enters intracellular fluid as the anion, a positive intracellular ion is necessary (i.e., potassium). If the organic acid entered as the intact molecule, displacement of an intracellular cation would not be necessary. The slight increase in plasma potassium might be related to the observation that acute metabolic acidosis will lead to a decreased potassium excretion.

Fluid Shifts

The data from the fluid shift study and PCV data would seem to indicate that the infused solutions remained within the vascular compartment, leading to a dilution of the remaining blood volume. This dilution corresponds to a decrease in packed cell volume seen for all groups. The decrease in the PCV in the control group could be a result of a fluid shift - interstitial fluid to vascular compartment, and is consistent with known data.

CHAPTER VI

SUMMARY AND CONCLUSIONS

In conclusion, it appears that, for the correction of an acidosis produced by the removal of 20% of the measured blood volume of dogs, a solution containing a maintenance dose of sodium bicarbonate (24 mEq/l) plus a dose for the correction of the base excess (0.3 x body weight in kilograms) was the most efficient when compared to solutions containing sodium lactate and the untreated control group.

The index used to determine acidosis was the base excess and it was found that the groups receiving the bicarbonate solutions remained within the normal range of base excess for the observation period. The groups receiving the lactate solutions remained at the level of the untreated groups for the duration of the experiment. Elevation of the anion gap was the greatest for these latter groups. The PCO_2 and bicarbonate concentrations were lowest for the lactate and untreated groups. However, the bicarbonate was below normal for the animals receiving the bicarbonate solutions, perhaps indicating a dilution of the plasma bicarbonate by fluid administration. This would appear to indicate that the use of at least 25 mEq/l sodium bicarbonate is necessary to prevent a more severe dilution of the plasma buffer systems

following the administration of intravenous fluids. It should be noted that, although the animals receiving the lactate solutions responded in a similar manner as the untreated group, fluid replacement is still a priority in the treatment of hypovolemia.

Changes in plasma sodium, chloride and potassium were consistent with the changes that might occur in these parameters during an organic acid acidosis.

SELECTED BIBLIOGRAPHY

1. O'Shaughnessy, W. B. 1831-1832. Experiments on the blood in cholera. Lancet 1:490.

2. Latta, T. 1831-1832. Letter to the Secretary of the Central Board of Health, London, affording a view of the rationale and the results of his practice in the treatment of cholera by aquaeous and saline injections. Lancet 2:274.

3. Rose, R. J., and R. J. Carter. 1980. Intravenous fluid therapy for non-respiratory acidosis in dogs: A comparison of a balanced electrolyte solution with a fluid rich in potassium and bicarbonate. J. Vet. Pharm. Therap. 2:9-19.

4. Harmann, A. F., and M. J. E. Senn. 1932. Studies in the metabolism of sodium R-lactate. 1. Response of normal human subjects to the intravenous injection of sodium R-lactate. J. Clin. Invest. 11:327-335.

5. Mudge, G. H., J. A. Manning, and A. Gilman. 1949. Sodium acetate as a source of fixed base. Proc. Soc. Exp. Biol. Med. 71:136-138.

6. Cornelius, L. M. 1981. Fluid, Electrolyte, Acid Base and Nutritional Management, in <u>Pathophysiology in Small Animal Surgery</u>, M. J. Bojrab (Ed.), Lee and Febiger, Philadelphia, Pennsylvania.

7. Eliahou, H. E., P. H. Feng, U. Weinberg, A. Iaina, and E. Resin. 1970. Acetate and bicarbonate in the treatment of uraemic acidosis. British Med. J. 4:399-401.

8. Schumer, W. 1978. Cell Metabolism and Lactate, in <u>International Symposium on Lactate in Acute Conditions</u>, Basel (Ed.), p. 1.

9. Hartsfield, S. M., J. C. Thurmon, and G. J. Benson. 1981. Sodium bicarbonate and bicarbonate precurors for treatment of metabolic acidosis. JAVMA 179:914-916.

10. Hartsfield, S. M., J. C. Thurmon, J. E. Corbin, G. J. Benson, and T. Aiken. 1981. Effects of sodium acetate, bicarbonate and lactate on acid-base balance in anesthetized dogs. J. Vet. Pharm. Therap. 4:51-61.

11. Nelson, A. W. 1981. Shock: An Introduction in Pathophysiology in Small Animal Surgery, M. J. Bojrab (Ed.), Lea and Febiger, Philadelphia, Pennsylvania.

12. Gevers, W., and E. Dowdle. 1963. The effect of pH on glycolysis in vitro. Clin. Sci. 25:343.

13. Schwartz, W. B., and W. C. Waters. 1962. Lactate versus bicarbonate. Am. J. Med. 32:831-834.

14. Mithoefer, J. C., and M. S. Karetzky. 1968. Comparative effects of organic and mineral acid on acid base balance and gas exchange. J. Lab. Clin. Med. 72:924-932.

15. Berliner, R. W., and G. Giebish. 1979. Body Fluids and the Excretion of Urine, in Best and Taylor's Physiological Basis of Medical Practice, J. R. Brobeck (Ed.), William and Wilkins, Baltimore, Maryland.

16. Woodbury, D. M. 1974. Physiology of Body Fluids, in Physiology and Biophysics. II. Circulation, Respiration and Fluid Balance, T. C. Ruch, and H. D. Patton (Eds.), W. B. Saunders, Philadelphia, Pennsylvania, pp. 450-479.

17. Breazile, J. E. In publication. Physiological basis of fluid therapy. OVMA.

18. Breazile, J. E. 1981. Fluid and electrolyte physiology. OVMA 33:2-8.

19. Woodbury, J. W. 1974. Body Acid Base and Its Regulation, in Physiology and Biophysics. II. Circulation, Respiration and Fluid Balance, Ruch and Patton (Eds.), W. B. Saunders, Philadelphia, Pennsylvania, pp. 480-524.

20. Mudge, G. H. 1980. Water, Salts and Ions. Agents Affecting Volume and Composition of Body Fluids, in Goodman and Gilman's The Pharmacological Basis of Therapeutics, A. Gilman, and L. S. Goodman (Eds.), MacMillan, New York, New York, pp. 848-884.

21. Pitts, R. F. 1974. Physiology of the Kidney and Body Fluids, Yearbook Medical Publishers, Inc., Chicago, Illinois.

22. Pace, N., L. Kline, H. K. Schachman, and M. Harfenist. 1947. Studies on body composition. IV. Use of radioactive hydrogen for measurement in vivo of total body water. J. Biol. Chem. 168:459-469.

23. Soberman, R., B. B. Brodie, B. B. Levy, J. Axelrod, V. Hollander, and J. M. Steele. 1949. The use of antipyrine in the measurement of total body water in man. J. Biol. Chem. 179:31-42.

24. Dawson, A. B., H. M. Evans, and G. H. Whipple. 1920. Blood volume studies. III. Behavior of a large series of dyes introduced into the circulating blood. Am. J. Physiol. 51:232-256.

25. Chrystal, R. G., and A. E. Baue. . Influence of hemorrhagic hypotension on measurements of the extracellular fluid volume. Surg. Gynecol. Obstet.

26. Berson, S. A., and R. S. Yalow. 1952. The use of K^{42} or P^{32} labelled erythrocytes and I^{131} tagged human serum albumin in simultaneous blood volume determinations. J. Clin. Invest. 31:572-580.

27. Vidt., D. G., and L. A. Sapirstein. 1957. Distribution volumes of T1824 and chromium 51 labelled red cells immediately following injection. Circ. Res. 5:129-132.

28. Kim, S. I., J. M. Desai, and W. C. Shoemaker. 1969. Sequence of cardiorespiratory alterations after gradual prolonged hemorrhage in conscious dogs. Am. J. Physiol. 216(5):1044-1050.

29. Weiskopf, R. B., M. I. Townsley, K. K. Riordan, K. Chadwick, M. Baysinger, and E. Mahoney. 1981. Comparison of cardiopulmonary responses to graded hemorrhage during enflurane, halothane, isoflurane and ketamine anesthesia. Anesth. Analg. 60:481-491.

30. Chien, S. 1948. Quantitative evaluation of the circulatory adjustment of splenectomized dogs to hemorrhage. Am. J. Physiol. 193(3):605-614.

31. Wood, C. E., J. Shinsako, L. C. Kiel, D. J. Ramsay, and M. F. Dallman. 1981. Hormonal and hemodynamic responses to 15 ml/kg hemorrhage in conscious dogs: responses correlate to body temperature. Proc. Soc. Exp. Biol. Med. 167:15-19.

32. Swan, H., J. Blavier, T. Marchioro, D. Jenkins, and V. Montgomery. 1959. Experimental hemorrhage: prediction of mortality following acute measured hemorrhage in the dog. AMA Arch. Surg. 79:176-184.

33. Dillon, A. R., G. H. Hankes, R. F. Nachreiner, and R. W. Redding. 1980. Experimental hemorrhage in splenectomized and nonsplenectomized dogs. Am. J. Vet. Res. 41:707-711.

34. Swan, H. 1965. Experimental acute hemorrhage. Am. Med. Assoc. Arch. Surg. 91:390.

35. Breazile, J. E. In press. Cardiovascular physiology. The circulation of blood.

36. Meek, W. J., and J. A. E. Eyster. 1921. Am. J. Phsiol. 56:1.

37. Cope, O., and S. B. Litwin. 1962. Contribution of the lymphatic system to the replenishment of the plasma volume following a hemorrhage. Ann. Surg. 156:655-667.

38. Doberneck, R. C., D. G. Johnson, and R. M. Hardaway. 1963. Blood volume adjustments to shock in dogs. Arch. Surg. 86:267-271.

39. Hardaway, R. M. 1981. Expansion of the intravascular space in severe shock. Am. J. Surg. 142:258-261.

40. Relman, A. S. 1972. Metabolic consequences of acid base disorders. Kidney Inst. 1:347-359.

41. Oliva, P. 1970. Lactic acidosis. Am. J. Med. 48: 209-225.

42. Alberti, K. G. M., and M. Nathrass. 1977. Lactic acidosis. Lancet 2:25-29.

43. Cohen, R. D., and R. A. Iles. 1977. Lactic acidosis: some physiological and clinical considerations. Clin. Sci. Mol. Med. 53:405-410.

44. Montgomery, R., R. L. Dryer, T. W. Conway, and A. A. Spector. 1977. Biochemistry. A Case Oriented Approach, C. V. Mosby, St. Louis, Missouri.

45. Schumer, W., and P. R. Erve. 1975. Cellular metabolism in shock. Circ. Shock 2:109-127.

46. Cohen, R. D. 1978. The Production and Removal of Lactate, in International Symposium on Lactate in Acute Conditions, Basel.

47. Iles, R. A., R. D. Cohen, A. H. Rist, and P. G. Baron. 1977. The mechanism of inhibition of gluconeogenesis from lactate in rat liver. Biochem. J. 164:185-191, 1977.

48. Wiener, R., and J. J. Spitzer. 1973. Lactate metabolism following severe hemorrhage in the conscious dog. Am. J. Physiol. 227:58-62.

49. Perret, C., and J. F. Enrico. 1978. Lactate in Acute Circulatory Failure, in International Symposium on Lactate in Acute Conditions, pp. 69-82.

50. Cohen, R. D., and R. Simpson. 1975. Lactate metabolism Anestheosiology 43:661-673.

51. Cohen, R. D., and H. F. Woods. 1976. Clinical and Biochemical Aspects of Lactic Acidosis, Blackwell Scientific Publications, Oxford.

52. Cohen, J. J., and J. P. Kassiere. 1982. Acid Base, Little Brown and Company, Boston, Massachusetts.

53. Feldman, B. F., and D. P. Rosenberg. 1981. Clinical use of anion and osmolal gaps in veterinary medicine. JAVMA 178:396-398.

54. Dell, R. 1973. Normal Acid Base Regulation, in The Body Fluids in Pediatrics, R. W. Winters (Ed.), Little Brown, Boston, Massachusetts, pp. 23-45.

55. Siggard-Andersen, O. 1964. The Acid Base Status of the Blood, Williams and Wilkins Co., Baltimore, Maryland.

56. Davenport, H. W. 1969. The ABC of Acid Base Chemistry, 4th ed., University of Chicago Press, Chicago, Illinois.

57. Stewart, P. A. 1981. How to Understand Acid Base. A Quantitative Acid Base Primer for Biology and Medicine. Elsevier, New York, New York.

58. Van Slyke, D. D., A. B. Hastings, A. Hiller, and J. Sendroy. 1928. Studies of gas and electrolyte equilibria in blood. XIV. The amounts of alkali bound by serum albumin and globulin. J. Biol. Chem. 79:769-780.

59. Houpt, T. R. 1977. Water,Electrolytes and Acid Base Balance, in Duke's Physiology of Domestic Animals, M. J. Swenson (Ed.), Cornell University Press, Ithaca.

60. Van Slyke, D. D., and J. Sendroy. 1933. Studies of gas and electrolyte equilibria in blood. XVII. The effects of oxygenation and reduction on the carbon dioxide absorption curve and the pK' of whole blood. J. Biol. Chem. 102:505-519.

61. Swan, R. C., and R. F. Pitts. 1955. Neutralization of infused acid by nephrectomized dogs. J. Clin. Invest. 34:205-212.

62. Oster, J. R., G. O. Perez, and C. A. Vaamonde. 1978. Relationship between blood pH and potassium and phosphorous during acute metabolic acidosis. Am. J. Physiol. 235:F345-351.

63. Androgue, H. J., and N. E. Madias. 1981. Changes in plasma potassium concentration during acute acid base disturbances. Am. J. Med. 71:456-467.

64. Hartsfield, S. M., J. C. Thurmon, J. E. Corbin, G. J. Genson, and T. Aiken. 1981. Effects of sodium acetate, bicarbonate and lactate on acid-base balance in anesthetized dogs. J. Vet. Pharm. Therap. 4:51-61.

65. Scribner, B. H., and J. M. Bunnell. 1956. Interpretation of the serum potassium concentration. Metabolism 5:468-479.

66. Fenn, W. O., and D. M. Cobb. 1935. Evidence for a potassium shift from plasma to muscles in response to an increased carbon dioxide tension. Am. J. Physiol. 112:41-55.

67. Fenn, W. O., D. M. Cobb. 1934. The potassium equilibrium in muscle. J. Gen. Physiol. 17:629-656.

68. Simmons, D. H., and M. Avedon. 1959. Acid base alterations and plasma potassium concentration. Am. J. Physiol. 197:319-326.

69. Kilburn, K. H. 1965. Movements of potassium during acute respiratory acidosis and recovery. J. Appl. Physiol. 21:679-684.

70. Lade, R. I., and E. B. Brown. 1963. Movement of potassium between muscle and blood in response to respiratory acidosis. Am. J. Physiol. 204:761;764.

71. Clancy, R. L., and E. B. Brown. 1963. Changes in bone potassium in response to hypercapnia. Am. J. Physiol. 204:757-760.

72. Spurr, G. B., and H. Lambert. 1960. Cardiac and skeletal muscle electrolytes in acute respiratory alkalemia and acidemia. J. Appl. Physiol. 15:459-464.

73. Keating, R. E., T. E. Weischelbaum, M. Alanis, H. W. Magraf, and R. Elman. 1953. The movement of potassium during experimental acidosis and alkalosis in the nephrectomized dogs. Surg. Gynecol. Obstet. 96:323-330.

74. Abrams, W. B., D. W. Lewis, and S. Bellet. 1951. The effect of acidosis and alkalosis on the plasma potassium concentration and the electrocardiogram of normal and potassium depleted dogs. Am. J. Med. Sci. 222:506-515.

75. Androgue, H. J., J. Brensilver, and N. E. Madias. 1978. Changes in the plasma anion gap during chronic metabolic acid base disturbances. Am. J. Physiol. 235:F291-297.

76. Oh, M. S., and H. J. Carroll. 1977. The anion gap. New Eng. J. Med. 297:814-817.

77. Emmett, M. E., and R. G. Narins. 1977. Clinical use of the anion gap. Medicine 56:38-54.

78. Koppel, M., and K. Spiro. 1914. Uber die wirkung von Moderatoren (Puffern) bei der Verschiebung des Saure-Basengleichgewichtes in biologischen Flussigkeiten. Biochem. Z. 65:409.

79. Henderson, L. J. 1908. The theory of neutrality regulation in the animal organism. Am. J. Physiol. 21:437.

80. Mendelson, J. A. 1981. Choice of fluids for resuscitation in trauma. Military Medicine 146:677-681.

81. Blood Component Therapy, A Physicians Handbook. Second Edition, American Association of Blood Banks, Washington, D. C., 1975, p.i.

82. Gambino, S. R., W. Burns, and J. O'Brien. 1967. The solution is the problem. Current Medical Digest 34:1613-1619.

83. Lane, W. A. 1891. A surgical tribute to the late Dr. Wooldridge. Lancet 2:620-627.

84. Filley, G. F. 1972. Acid Base and Blood Gas Regulation, Lea and Febiger, Philadelphia, Pennsylvania.

85. Stewart, J. D., and H. M. Rourke. 1942. The effects of large intravenous infusions on body fluid. J. Clin. Invest. 21:197.

86. Garella, S., B. S. Chang, and S. I. Kahn. 1975. Dilution acidosis and contraction alkalosis: review of a concept. Kidney Int. 8:279-283.

87. Shires, G. T., and J. Holman. 1948. Dilution acidosis. Ann. Intern. Med. 28:557-5591

88. Wiener, R., and J. J. Spitzer. 1973. Lactate metabolism following severe hemorrhage in the conscious dog. Am. J. Physiol. 227:58-62.

89. Osano, E., E. Koto, M. Yamaushi, Y. Ozawa, M. Isawa, T. Wada, and H. Hasegawa. 1966. The mechanism of the acidosis caused by infusion of saline infusion. Lancet 1:1245.

90. Rosenbaum, B. J., D. I. Makoff, and M. H. Maxwell. 1969. Acid base and electrolyte changes induced by acute isotonic saline infusion in the nephrectomized dog. J. Lab. Clin. Med. 74:437-435.

91. Brasmer, T. H. 1979. Fluid therapy in shock. JAVMA 174: 475-478.

92. Brown, W. J., B. S. Kim, D. B. Weeks, and C. E. Parkin. 1978. Physiologic saline solution, Normosol R pH 7.4 [R] and Plasamanate as reconstituents of packed human erythrocytes. Anthesiology 49:99-101.

93. Kirkendol, P. L., J. Starrs, and F. M. Gonzalez. 1980. The effects of acetate, lactate, succinate and gluconate on plasma pH and electrolytes in dogs. Trans. Am. Soc. Artif. Intern. Organs 26:323-326.

94. Trinkle, J. K., B. F. Rush, and B. Eiseman. 1968. Metabolism of lactate following major blood loss. Surgery 63(5):782-787.

95. Huckabee, W. E. 1961. Abnormal resting blood lactate. I. The significance of hyperlactatemia in hospitalized patients. Am. J. Med. 30:833-839.

96. Phillipson, E. A., and B. J. Sproule. 1965. The clinical significance of elevated blood lactate. Can. Med. Assoc. J. 92:1334.

97. Clausen, S. W. 1925. Anhydremic acidosis due to lactic acid. Am. J. Dis. Child. 29:761.

98. Rush, B., and B. Eiseman. 1967. Limits of noncolloid solution replacement in experimental hemorrhagic shock. Ann. Surg. 165:977-984.

99. Canizaro. P. D., M. D. Prager, and G. T. Shires. 1971. The infusion of Ringer's lactate solution during shock: Changes in lactate, excess lactate and pH. Am. J. Surg. 122:494-501.

100. Garella, S. C., L. Dana, and J. A. Chazan. 1973. Severity of metabolic acidosis as a determinant of bicarbonate requirements. New Eng. J. Med. 289:121-126.

101. Mellemgard, K., and P. Astrup. 1960. The quantitative determination of surplus amounts of acid base in the human body. Scand. J. Clin. Lab. Invest. 12:187-199.

APPENDIXES

ANTIPYRINE ANALYSIS

1. Unknown determination is made with plasma.
2. Separate plasma.
3. Add: 1 ml cadmium sulfate reagent

   1 ml 4.4% NaOH

   to 2 ml plasma. Stir, then centrifuge.
4. Label three tubes: BLANK STANDARD TEST
5. The Standard tube contains: 0.2 mg antipyrine

   The Text tube contains: 1.5 ml protein free filtrate
6. Add: 5 ml glacial acetic acid

   0.25 ml of 15% sodium nitrite

   to all three tubes.
7. Let stand for 20 minutes.
8. To all tubes add:

   0.25 ml ammonium sulfamate

   Shake well.

   1 ml 10% 1-napthylamine solution in glacial acetic acid

   Heat @ 50°C for 5 minutes.

   Cool; let stand for 20 minutes.
9. Read absorbance at 580 nm.

POTASSIUM THIOCYANATE ANALYSIS

1. 1 ml of plasma + 1 ml 10% Trichloroacetic acid
2. Shake, then centrifuge.
3. 1 ml of the supernatant + 1 ml distilled water + 1 ml 5% $Fe(NO_3)_3$ in HNO_3
4. Read at 460 nm.
5. Extracellular fluid space + 0.7 x thiocyanate space

2

VITA

Howard Jeffrey Mass

Candidate for the Degree of

Doctor of Philosophy

Thesis: A STUDY OF THE TREATMENT OF METAPOLIC ACIDOSIS IN DOGS

Major Field: Physiological Sciences

Biographical:

Personal Data: Born in Brooklyn, New York, April 18, 1954, the son of Gerald and Lillian Mass.

Education: Graduated from Lenape High School in May, 1972; received the Bachelor of Arts degree from University of Delaware in May, 1976, with a major in Biology; received the Master of Science degree at Oklahoma State University, July, 1980, with a major in Physiological Sciences; completed requirements for the Doctor of Philosophy degree with a major in Physiological Sciences at Oklahoma State University, May, 1984.

Professional Experience: Science Associate, Department of Physiology, Texas College of Osteopathic Medicine, September, 1982 - present; teaching assistant, Department of Physiological Sciences, Oklahoma State University, September, 1977 to May, 1980; teaching Introductory Physiology Laboratory and Veterinary Physiology Laboratory; laboratory assistant in the Department of Physiological Sciences, Oklahoma State University, May, 1979 to August, 1979.